# EXERCISE TESTING, LIMITATIONS AND TRAINING IN PATIENTS WITH CYSTIC FIBROSIS

## A personalized approach

**Maarten S. Werkman**

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**Exercise testing, limitations and training in patients with cystic fibrosis:**
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# EXERCISE TESTING, LIMITATIONS AND TRAINING IN PATIENTS WITH CYSTIC FIBROSIS

## A personalized approach

**Inspanningstesten, inspanningsbeperking en training bij patiënten met Cystic Fibrosis**

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# Chapter 1

# General introduction

Maarten S. Werkman

## PATHOPHYSIOLOGY IN PATIENTS WITH CYSTIC FIBROSIS

Cystic fibrosis (CF) or mucoviscidosis is the most common inherited disease in Caucasians with a current mean life expectancy below 40 years of age.[1] However, with the introduction of newborn screening and continuously improving evidence-based medicine the expected median survival for babies born in the 21st century is over 50 years.[2]

CF is caused by a mutation on the long arm of chromosome 7, which encodes for a protein product called the CF transmembrane conductance regulator (CFTR), which acts as an epithelial chloride channel. CFTR is important to enable flow of electrolytes and fluids across cellular membranes. CFTR dysfunction results in impaired electrolyte transport, resulting in dehydrated, and therefore, more viscous mucus. The CFTR defect can be classified in 5-6 classes, based on the type of the genetic defect. It ranges from complete loss of protein synthesis to only minimal dysfunction, which affects the degree of clinical severity.[3]

The negative effects of the viscous mucus mainly affect the pancreas, bowels, reproductive tracts and, above all, the lungs. The clinical presentation of CF varies with age.[2] In particular, in most patients the airways are chronically obstructed with mucus. This impairs breathing and results in overgrowth of bacteria and other infectious agents. The resulting chronic inflammation leads to progressive destruction of the airways, thereby deteriorating lung function.[3] Further clinical features of CF are chronic cough and wheeze, static and/or dynamic hyperinflation, tachypnea and radiographic abnormalities as bronchiectasis.[4] Eventually, in most patients, respiratory insufficiency results, this is the direct cause of death in most patients.[3] (Fig. 1)

Although the main clinical manifestations are noted in the respiratory tract, other tracts and systems become affected primary or secondary to CF. Intestinal malabsorption and pancreatic insufficiency occurs, leading to steatorrhea and failure to thrive. Malabsorption can cause malnutrition with decreased muscle mass.[2, 3] Furthermore, lack of CFTR in skeletal muscles might predispose to skeletal muscle weakness, as reported in CF mice.[5]

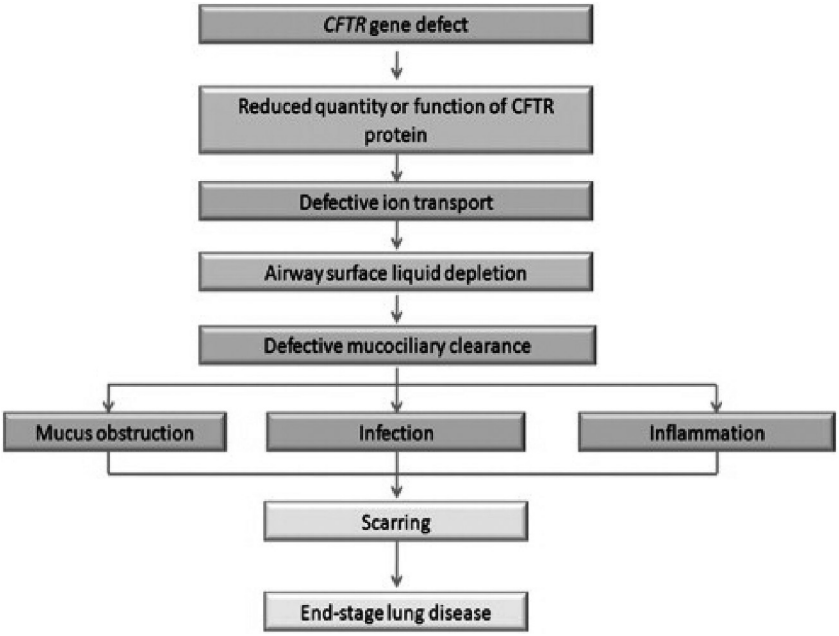


**Figure 1.** Pathophysiological cascade of CF lung disease [3]

**Exercise capacity and exercise limiting mechanisms in cystic fibrosis**

Exercise capacity is traditionally represented by the peak oxygen uptake ($VO_{2peak}$). $VO_{2peak}$ is determined by the maximal uptake of oxygen in the lungs, the oxygen transport capacity of the cardiovascular system, and the maximum oxygen extraction rate of the cells (during exercise mainly the exercising skeletal muscles).[6] The Wasserman wheels (Figure 2) provide an overview of the normal coupling between the musculoskeletal, the cardiovascular and the respiratory systems during exercise. Disruption of the normal coupling between these systems during exercise might lead to limited exercise capacity, especially with progressive disease.

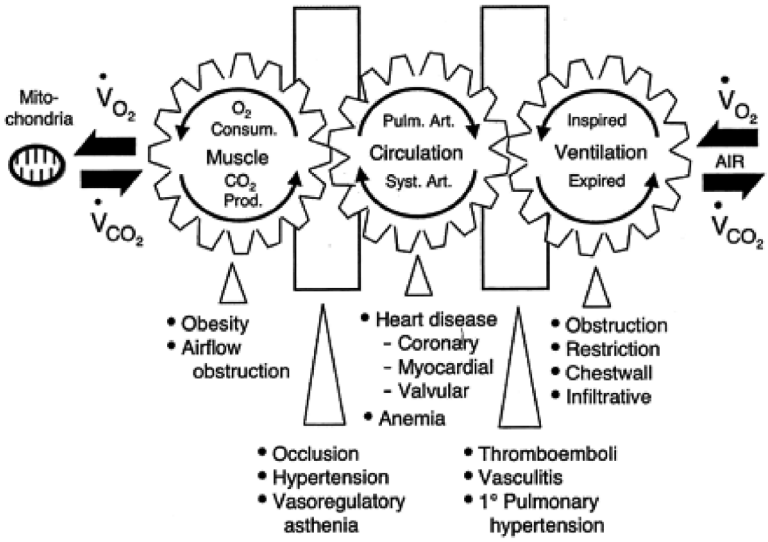


**Figure 2.** Sites of interference in the metabolic-cardiovascular-ventilatory coupling for various disease states [6]

Many patients with CF have a decreased $VO_{2peak}$, which may be caused by multiple factors. [7, 8] Respiratory, cardiovascular and peripheral muscle function, are all reported as potential exercise limiting mechanisms.[7] The $VO_{2peak}$ has been demonstrated to be a significant predictor of mortality, both as percentage of predicted [9, 10] or as absolute value ml/min/kg.[11] Compared with age matched healthy controls the $VO_{2peak}$ of patients with CF declines from 100% of predicted at the age of twelve to 80% of predicted during adolescence. In a recent study from our center it was showed that this longitudinal decline in $VO_{2peak}$ was negatively associated with chronic Pseudomonas aeruginosa infection and systematic inflammation, but independent of age, pulmonary function and body mass. [12]

## Respiratory factors

Pulmonary function is moderately associated with aerobic and anaerobic exercise capacity. [13, 14] An early clinical feature of lung disease is the development of hyperinflation, which increases with progressive lung destruction. Progressive hyperinflation changes the shape of the thorax, putting the inspiratory muscles (and particularly the diaphragm) at a mechanical disadvantage.[15] This increases work of breathing in rest and during exercise. These changes may compromise respiratory muscle function; patients with CF are thus more susceptible to respiratory failure or ventilatory limitation during exercise. [16] During sub-maximal and strenuous exercise, work of breathing is relevantly higher

in children with CF compared to healthy controls.[17] Subsequently, the increased work of breathing may increase the oxygen cost of the respiratory muscles up to ~10-15% of total $O_2$-consumption.[18] This "oxygenation competition" limits the blood flow available for locomotor muscles and thereby limits their (aerobic) work capacity. This phenomenon of blood flow competition is referred to as the "respiratory muscle induced metaboreflex". [19] Respiratory muscle fatigue causes a reflex vasoconstriction of the blood flow through the locomotor muscles.[19] Nevertheless, maximal exercise capacity is generally not ventilatory limited until the forced expiratory volume in one second ($FEV_1$ (L) is less than 60% predicted.[7]

## Cardiac factors

A decreased cardiac stroke volume has been described in several studies in patients with CF, although this is most often asymptomatic. It may be associated with malnutrition,[20] but might also be caused by the occurrence of both right and left ventricular dysfunction during exercise.[21] A postmortem study showed evidence of right ventricular (RV) hypertrophy in 70% of children with CF,[22] which was confirmed in vivo in both clinical and non-clinical stable patients with CF.[23, 24, 25] This RV dysfunction may also be secondary to pulmonary hypertension, chronic hypoxemia [26, 27] or the presence of chronic systemic inflammation.[25] Still, the exact prevalence of sub-clinical RV dysfunction in the CF population is unknown, but prognosis is poor once RV failure is (clinically) manifest.[28] Evidence for left ventricular (LV) dysfunction in patients with CF remains scarce,[24, 25, 29] but there is some evidence for LV dysfunction [28] possibly associated with cardiac muscle CFTR dysfunction.[30] LV dysfunction seems not to be of major importance in mild disease state, but, next to other previously mentioned factors, may become clinically evident in more progressive disease and eventually limit cardiac output during exercise.[31]

## Peripheral skeletal muscle factors

Malnutrition can induce loss of muscle mass,[32] leading to reduced peripheral muscle strength.[33-38] Peripheral muscle strength is significantly associated with $FEV_1$.[36, 37] Furthermore, peak anaerobic capacity,[39, 40] maximum work capacity [41] and, to a lesser extend, aerobic capacity [14] are also related to fat free mass. Preliminary results also suggest mild impaired twitch contractile properties causing skeletal muscle fatigue in patients with CF.[35] Although systemic inflammation in patients with CF is suggested to be related to reduced muscle strength,[33] it does not seem to be an independent predictor of respiratory and limb muscle strength.[42] Besides, recently, the expression

of CFTR protein in human skeletal muscle cells has been demonstrated.[5, 43] This is in agreement with a number of studies reporting evidence for intrinsically impaired skeletal muscle function in CF patients, independent of lung function and/or muscle mass.[44-47] In addition, there is some evidence for altered proton handling and reduced mitochondrial function in CF muscles.[44] A recent study reported attenuated mitochondrial function in CFTR deficient non-skeletal muscle cells.[48] There is still no consensus about either its exact localization in the muscle cell or any potential impact of a mutated CFTR protein on muscular contractile performance during exercise.[44-47, 49, 50] More exercise studies are warranted to resolve this debate.

Furthermore, impaired skeletal muscle function may also be caused by poor oxygenation of the skeletal muscles, possibly due to impaired blood flow ("steal effect") during exercise with excessive ventilatory demands,[18] as described earlier in this chapter. Studies focusing on the oxygenation during exercise are necessary to provide insight in this possible mechanism.

Finally, respiratory gas exchange ratio's ($VCO_2/VO_2$), both at rest and during submaximal exercise seem to be higher in patients with CF.[46, 51] This could be explained by impaired fat metabolism and relatively high rates of carbohydrate oxidation during (sub)maximal exercise as recently reported in boys with CF.[52]

In conclusion, in patients with mild to moderate pulmonary disease, non-pulmonary factors, such as low muscle mass and impaired skeletal muscle function, predominate in limitation of exercise capacity.[53, 54] In more severe patients with CF (~$FEV_1$ < 60%pred), ventilatory constraints and impaired gas exchange become more important determinants. However, in mild, moderate and severe lung disease other factors also influence exercise capacity, e.g. CF specific skeletal muscle defects and/or systemic inflammation. Unique specific combinations of all these factors might limit exercise capacity in the individual patient.

This implicates that exercise training in each patient should focus on personalized exercise interventions. These distinctive inter-individual characteristics require detailed cardiopulmonary assessment and exercise testing prior to the prescription of exercise training in order to provide the patients with personalized, feasible, effective and safe training programs.[55]

## EXERCISE TESTING IN CF

Cardiopulmonary exercise testing (CPET) plays an integral role in the follow up of patients with CF because of its high-yield of diagnostic, prognostic and functional information. In addition, it can be used as screening for possible adverse effects of exercise.[56] One of the most important CPET outcome parameters is $VO_{2peak}$,[57-59] commonly defined as the highest oxygen uptake attained during a single test without necessarily achieving a plateau of the $VO_2$ curve.[60] As mentioned previously, the $VO_{2peak}$ is a significant predictor of mortality in CF, both as percentage of predicted [9, 10] or as absolute value,[11] where a $VO_{2peak} < 32$ ml·min·kg$^{-1}$ was associated with a 10 year mortality of 50%, whereas patients with a $VO_{2peak} > 45$ ml·min·kg$^{-1}$ showed a 100% 10 year survival.[11]

The Clinical Practice Guideline of Exercise Testing in Cystic Fibrosis recommends the Godfrey cycle ergometer protocol with monitoring of oxygen saturation and ventilatory gas exchange for routine monitoring in people 10 years and older.[61] This Godfrey protocol [62] is a validated procedure designed to induce exhaustion within 10 to 12 minutes and is frequently used in patients with CF.[63] Despite the clinical value, many specialized CF centers still do not perform maximal exercise testing. A recent survey indicated that the majority of UK CF clinics do not have the resources to directly measure $VO_{2peak}$ (availability of metabolic gas analysis system with treadmill or cycle ergometer). Apart from laboratory tests, field tests (e.g. 6 minute walk test / Shuttle tests / Step-tests) are frequently used to assess exercise capacity because they offer a simple and inexpensive means of estimating exercise capacity.[64, 65] Regrettably, recent evidence suggests that the commonly used 6-minute walk test is not very well associated with $VO_{2peak}$ in children and adolescents with CF [66] and that field-tests often miss important clinical information due to their relatively low intensity.[55]

As peak workload ($W_{peak}$) and $VO_{2peak}$ during cycle ergometry are strongly related in adolescents with CF,[41] a valid and inexpensive cycle ergometer exercise test without the necessity of direct gasanalysis, may help to increase the applicability of exercise testing in both daily practice and research of this patient group.[64, 65, 67, 68] The large differences in disease severity and the progressive nature of CF make a more individualized or personalized approach to exercise tests critical. To date, it is generally accepted that there is no single best exercise testing protocol to answer all questions in individuals with CF.[64, 69] Ultimately, the characteristics of the patient, the disease severity and the purpose of the exercise test should determine which protocol is most suited for each individual patient.

## EXERCISE TRAINING IN CF

Since long, exercise training to optimize, stabilize of improve exercise capacity, has been considered an important component of the management of patients with CF.[8] Although importantly related to mortality, individualised exercise training programmes still seem to be underused in CF clinics (15-22%).[65]

Both children and adults with CF are able to increase exercise capacity with exercise training, regardless of disease severity.[8] Exercise training can impact positively on aerobic fitness, skeletal and respiratory muscle strength, mucus clearance, bone health, glucose tolerance and quality of life.[70] Regular exercise training and higher intensity levels of physical activity have the potential, both in the short and long term, to attenuate the decline in lung function.[8, 70] Schneiderman reported this attenuating effect on lung function decline to be gender specific for girls.[71]

As shown in the Cochrane Review of exercise training for cystic fibrosis, the benefits obtained from physical training may be influenced by the frequency, intensity, type and total duration of training.[72] Exercise training can be devided into aerobic exercise, anaerobic exercise, strength training and combinations of these. In a systematic review, Van Doorn et al. reported that aerobic exercise significantly improves $FEV_1$ and $VO_{2peak}$ on the short term and that it has the potential to slow the decline in $FEV_1$ in the long term. [73] The evidence for anaerobic exercise benefit is scarce. However, it improved (although not significantly) $VO_{2peak}$ and $W_{peak}$ within a 6-12 weeks training program in children and adolescents with mild-moderate CF ($FEV_{1\%pred}$~75%pred).[74, 75] Furthermore, in a group of deconditioned patients with CF with severe pulmonary disease ($FEV_{1\%pred}$ 25.5±7.5%; Age 26.4±7.5 years), six weeks of anaerobic training (high-intensity interval exercise) improved $VO_{2peak}$, $W_{peak}$ and peak minute ventilation ($VE_{peak}$).[76]

Strength training is even less explored in patients with CF. After a strength training regime two studies reported improved $FEV_1$ on the short term, but no attenuation of lung function decline on the longer term. Not surprising, significant gains in muscle strength were obtained after short [77] and long term [78] strength training.[77, 78]

Finally, several studies have focused on the effects of inspiratory muscle training (IMT) in patients with CF to increase maximal inspiratory muscle strength ($PI_{max}$), but the benefits are supported by weak evidence.[79, 80] Moreover, the impact of IMT in patients with CF on (sub-maximal) exercise capacity remains even more inconsistent,[79, 80] especially when considered as an addition to general exercise training.[81] As inspiratory muscle training has been shown to attenuate the previously mentioned reflex vasoconstriction with fatiguing breathing tasks in healthy controls,[82] this suggests that IMT could decrease work of breathing, which makes the inspiratory muscles more fatigue resistant. Less fatigable inspiratory muscles might improve exercise capacity and/or trainability of the locomotor skeletal muscles.

## AIMS AND RESEARCH QUESTIONS ADDRESSED IN THIS THESIS

This thesis consists of three parts. The first part covers studies investigating methodologies of clinical exercise testing methods in patients with CF. This part comprises three different studies in adolescents with CF, describing two alternative methodologies to measure exercise capacity without gas analysis and one study describing a verification method of the peak oxygen uptake measured with a standard exercise test protocol.
The aims of this part of the thesis were:

- To verify the $VO_{peak}$ measured with traditional CPET using a supramaximal exercise protocol (*Chapter 2*)
- To develop a CF-specific, individualized approach to determine workload increments for a cycle ergometry testing protocol (*Chapter 3*);
- To predict peak oxygen uptake ($VO_{2peak}$) from the peak work rate ($W_{peak}$) obtained during a cycle ergometry test using the Godfrey protocol in adolescents with CF (*Chapter 4*)
- To assess the accuracy of $VO_{2peak}$ prediction model for prognostication clustering (*Chapter 4*).

The second part of this thesis focuses on possible exercise limiting mechanisms in patients with CF. One study focuses on static hyperinflation as exercise limiting factor, the other study focuses on the possible role of skeletal muscle dysfunction.
Specific aims of this part of the thesis are:

- To investigate whether static hyperinflation makes adolescents with CF more prone to a ventilatory limitation during exercise (*Chapter 5*)
- To evaluate if the amount of static hyperinflation (RV/TLC (%)) is a stronger predictor of exercise capacity than the degree of airflow obstruction $FEV_1$ (%pred) (*Chapter 5*)
- To test the hypothesis that abnormalities in oxygenation and/or muscle oxidative metabolism contribute to exercise intolerance in adolescents with CF (*Chapter 6*).

The third part of the thesis focuses on personalized exercise training in patients with CF, especially concerning training of the inspiratory and/or skeletal muscles.
The aim of this part is:

- To analyze the effects of a six-week home-based inspiratory muscle training program on respiratory factors (*Chapter 7*)
- To analyze if a six week, unsupervised home-based peripheral muscle training program increases exercise capacity (*Chapter 7*)
- To test if inspiratory muscle training prior to a peripheral muscle training program has a preconditioning effect (*Chapter 7*).

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Chapter
1

# Chapter 2

# Supramaximal verification of peak oxygen uptake in adolescents with cystic fibrosis

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## ABSTRACT

*Background*: The purpose of this study was to investigate whether the peak oxygen uptake ($VO_{2peak}$) attained in traditional cardiopulmonary exercise testing (CPET) in adolescents with CF could be verified by a supra-maximal exercise test.
*Methods:* Sixteen adolescents with CF ($FEV_1$%predicted [range 45-117%]) volunteered and successively performed CPET and a supra-maximal test (Steep Ramp Test protocol (SRT)).
*Results:* CPET and the SRT resulted in comparable cardio respiratory peak values. We found no significant difference in $VO_{2peak}$/kg between CPET and SRT (38.9 ± 7.4 and 38.8 ± 8.5 $mL \cdot min^{-1} \cdot kg^{-1}$, respectively; $p = .81$). We found no systemic bias for CPET and SRT measurements of $VO_{2peak}$/kg and no differences between CPET and SRT $VO_{2peak}$ values within and between the maximal and non-maximal effort group ($p > .4$).
*Conclusion:* The $VO_{2peak}$ measured in CPET seems to reflect the true $VO_{2peak}$ in adolescents with CF.

## INTRODUCTION

Exercise testing is increasingly used to evaluate the level of exercise capacity and to define training intensity in adolescents with chronic lung diseases like cystic fibrosis (CF).[1, 2] Cardiopulmonary exercise testing (CPET) is currently accepted as golden standard to study a patient's aerobic capacity, and the possible limiting factors.[2, 3] Most clinical exercise testing is performed with progressive workloads during cycle ergometer or treadmill exercise. During both settings, cardioventilatory parameters as peak oxygen uptake ($VO_{2peak}$), peak workload ($W_{peak}$), peak heart rate ($HR_{peak}$) and the ratio of carbon dioxide production to oxygen consumption (respiratory exchange ratio = RER) can be calculated using gas-analysis of expired air.[4] The most important parameter of exercise capacity is the $VO_{2peak}$.[2, 3, 5] The maximum oxygen uptake ($VO_{2max}$) is considered to be the maximum attainable oxygen uptake by the cardiorespiratory and neuromuscular system, resulting in a $VO_2$ plateau at the end of testing despite further increase in workload.[6, 7] Furthermore, the $VO_{2peak}$ is defined as the highest level of oxygen uptake attained during a single test without necessity of a plateau of the $VO_2$ curve.[8] Questions can be raised about the validity of that attained $VO_{2peak}$ during CPET in adolescents with CF, because reduced exercise capacity during CPET in adolescents with CF compared to healthy peers are reported.[9-12] However the observed peak heart rates in these studies were lower compared to values observed in healthy adolescents. Therefore this lower $VO_{2peak}$ might be due to an actual lower $VO_{2peak}$ or to an incapability of the CEPT to reach real $VO_{2peak}$ in adolescents with CF. This possible inconsistency might influence the effectiveness of exercise training while in general, training intensity is defined based on CPET.

Whether this attained $VO_{2peak}$ reflects the true $VO_{2peak}$ could be verified by a supra-maximal exercise test following CPET,[7, 8, 13, 14] where supra-maximal means a workload above the peak workload attained during CPET. A feasible and safe supra-maximal exercise protocol is the Steep Ramp Test (SRT), which has been developed and described as an alternative measure of exercise work rate in adult patients with chronic heart failure [15-17] and adult cancer survivors.[18] Important difference of the SRT compared to CPET is its short duration (about 3-4 minutes including warming-up),[18] whereas the exercise time of the CPET will take on average 10-15 minutes. If consistent $VO_{2peak}$ values are found in both exercise tests, this provides support that a true $VO_{2peak}$ has been attained.[5]

Our hypothesis is that, based on lower peak heart rates in adolescents with CF, no actual $VO_{2peak}$ is reached in this population during CPET, resulting in higher $VO_{2peak}$ during the SRT which was conducted after CPET. The objective of this investigation was to verify the $VO_{2peak}$ attained during CPET in adolescents with CF using an additional supra-maximal exercise test (the SRT).

# METHODS

## Participants

Sixteen adolescents with CF (8♂ and 8♀; age 14.6 ± 1.7 years) volunteered. Patients participated in a study which was approved by the medical ethics committee of the University Medical Center Utrecht. All patients were free from acute exacerbation at the time of testing. Patients and their parents gave written informed consent. Only the initial baseline tests before exercise training were used for analysis. Exercise testing is part of the standard follow-up in the UMC Utrecht CF centre, so patients have experience with this kind of exercise testing.
Individual data were collected in one test session. Lung function (Master Lab system, E. Jaeger, Würzburg, Germany) and anthropometric values, using an electronic scale (Seca, Birmingham, UK) and a stadiometer (Ulmer stadiometer, Prof. E. Heinze, Ulm, Germany), were determined before CPET. While, due to planning, the exercise tests were performed in the morning, participants were asked to avoid heavy meals and strenuous exercise from the evening before testing (12 hours before testing).

## Cardiopulmonary exercise testing

CPET was performed on an electronically braked cycle ergometer (Ergoline, Cardinal Health, Houten, The Netherlands) using the Godfrey protocol.[19] In order to avoid premature muscle fatigue in the adolescents, we aimed to keep total exercise time between 6 and 10 minutes. Protocols with short-stage duration, as the Godrey protocol, are preferred if the test is conducted to measure performance.[20] After one minute of resting, cycling started unloaded and was increased based on height (10 W/min < 120 cm; 15 W/min 120 – 150 cm; 20 W/min > 150 cm), independent of gender, every minute until the patient stopped due to volitional exhaustion.[19] Adolescents breathed through a mouthpiece, connected to a calibrated metabolic cart (Oxycon pro, Care Fusion, Houten, The Netherlands). Expired gas passed through a flow meter, oxygen analyzer, and a carbon dioxide analyzer. The flow meter and gas analyzer, which were calibrated prior to each test session, were connected to a computer, which calculated breath-by-breath minute ventilation (VE), oxygen consumption ($VO_2$), carbon dioxide production ($VCO_2$), and respiratory exchange ratio (RER) from conventional equations. Breathing reserve (BR) was calculated as 1-(peak minute ventilation ($VE_{peak}$) / maximal voluntary ventilation (MVV)), where MVV is calculated as 37.5*$FEV_1$ (L·min)). While testing, heart rate (HR) was monitored continuously by a 3-lead electrocardiogram (Hewlett-Packard, Amstelveen, Netherlands), and transcutaneous oxygen saturation ($SpO_2$%) was measured by pulse oximetry on the index finger (Nellcor 200 E, Breda, The Netherlands). Heart rate response

(HRR) was calculated as [($HR_{peak}$ - $HR_{rest}$) / ($VO_{2peak}$ – $VO_{2rest}$)].[21] Data were collected from one minute rest throughout the entire test and data were averaged and presented over 10 seconds time intervals. Peak exercise parameters were defined as the values achieved at the final 30 seconds prior to stopping.

### Steep Ramp Test

The Steep Ramp test (SRT) was performed after maximal 10 minutes passive and subjective recovery following the CPET and was performed on the same electronically braked cycle ergometer. A trained physical therapist (MW) carrying out the tests made sure that continuing testing was safe (based on recovery of $SpO_2$ and absence of subjective signs of excessive cardiac or ventilatory stress). A modified protocol of the SRT was used.[22] The protocol was as follows: after one minute of resting and one minute of unloaded cycling, the test started with an increase in workload every 10 seconds based on the subject's height as in CPET. The test ended when the pedal frequency fell below 60 rpm despite verbal encouragement. Exercise parameters were measured and presented using the same methodology as during CPET. Peak exercise parameters were defined as the values achieved during the last 10 seconds before stopping. $W_{peak}$ was defined as the highest achieved work rate prior to stopping.

### Definition of maximal effort

We used previously described criteria in our laboratory for the definition of maximum exercise effort.[8] These criteria are subdivided into subjective and objective criteria. Subjective criteria are described as "unsteady biking", "sweating", "facial flushing" and "clear unwillingness to continue despite encouragement". Objective criteria are: [1] HR > 95% $HR_{predicted}$ (210-age), [2] RER > 1.00 and [3] Oxygen uptake plateau in last minute. The $VO_2$ plateau was determined from the difference between normalized $VO_{2peak}$ and $VO_2$ in the last 30 seconds of the minute before finish. When the difference was 2.1 $mL{\cdot}kg^{-1}min^{-1}$ or less, the adolescent was considered to have reached a plateau in $VO_2$.[23] These objective criteria are designed tot validate that participants optimally stressed their cardiopulmonary system. A participant has to meet the subjective criteria and at least 2 of the objective criteria for the test to be considered of maximal effort and character.[8]

### Statistical analysis

Data were expressed as mean ± SD. Data were analyzed using SPSS 15.0 for Windows (SPSS Inc, Chicago, Ill, USA) and tested for normality with the Kolmogorov-Smirnov Test. A p-value of <0.05 was considered statistically significant. Differences between CPET

and the SRT were analyzed using one-way repeated measures ANOVA and between the effort-groups with paired sample t-test. Associations were examined by Pearson product-moment correlation coefficient (r). Agreement between CPET and the SRT $VO_{2peak}$ was verified with the Bland and Altman method.[24] Association between the difference and average $VO_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$) [(CPET $VO_{2peak}$ + SRT $VO_{2peak}$) / 2)] was examined by Pearson product-moment correlation coefficient (r).

## RESULTS

### Participants

All (n = 16), except one, participants performed both exercise tests without any complications or adverse events. The one participant refused to do the SRT, because of subjective feelings of fatigue. In both tests, all participants indicated that their reason to stop the exercise test was leg muscle exhaustion / fatigue. Descriptive baseline characteristics are presented in Table 1. Of three patients the SRT gas-exchange data were missing due to software malfunction.

**Table 1.** Study group demographics

| Variable (n = 16) | Value (mean ± SD [range]) |
|---|---|
| Age (years) | 14.6±1.7 [12.1-17.4] |
| Weight (kg) | 50.1±10.5 [30.6-67.6] |
| Height (cm) | 166.3±13.6 [143.3-187.7] |
| Gender | 8 ♀; 8 ♂ |
| CFTR mutation | Homozygote ΔF508 |
| *Chronic Pseudomonas aeruginosa* infection* | "yes" n = 9; "no" n = 4; "intermittend" n = 3 |
| $FEV_1$%predicted ($FEV_1$ (L)) | 81±22 [45-117] (2.6±1.0) |

Legend: Values are means ± SD [range]. Abbreviations: CFTR = cystic fibrosis transmembrane conductance regulator; $FEV_1$ = forced expiratory volume in 1 second; * According to Leeds criteria (Lee et al, 2003): Yes = When more than 50% of months, when samples had been taken, were P. aeruginosa culture positive; No = No growth of P. aeruginosa during the previous twelve months, having previously been P. aeruginosa culture positive; Intermittent = When 50% or less of months, when samples had been taken, were P. aeruginosa culture positive.

### Comparison of resting and peak exercise variables between CPET and SRT

All exercise variables were normally distributed. Resting HR and resting VE were significantly ($p < .01$) higher in the SRT compared to CPET, whereas resting RER ($p < .01$) was significantly lower in the SRT compared to the CPET. No significant difference was noted for resting $VO_2$ ($ml \cdot min^{-1} \cdot kg^{1}$).

The mean exercise time of CPET was 11.0 ± 2.8 minutes and 4.1 ± 0.7 minutes for the SRT, both including one minute of resting measurements and one minute of reference cycling. Participants reached statistical significant ($p < .01$) higher $W_{peak}$ values in the SRT compared to the CPET, whereas $RER_{peak}$ and HRR were significantly ($p < .01$) lower during the SRT. No other statistical significant differences in cardiorespiratory variables were found between CPET and SRT at peak exercise (Table 2).

Additionally, oxygen consumption for comparable workloads in both tests seems to be less in the SRT. (Figure 1)

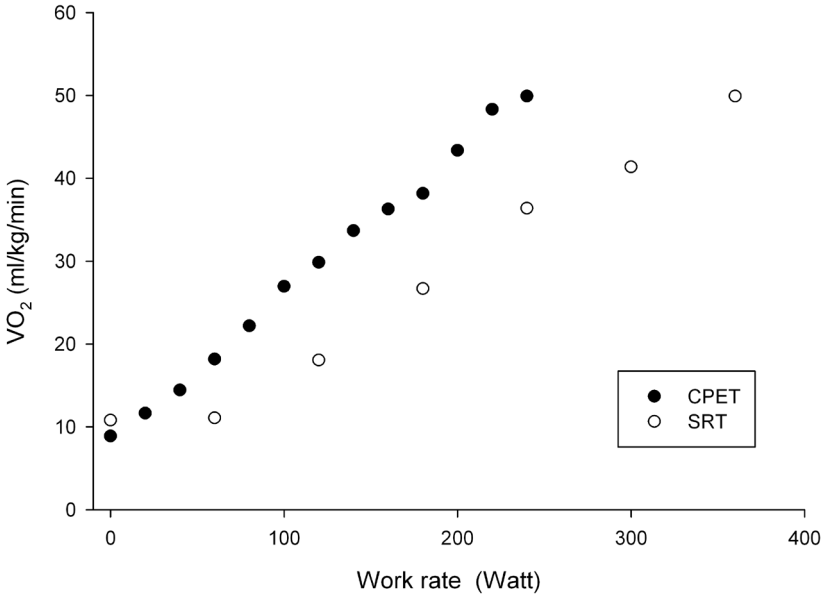


**Fig. 1.** Oxygen consumption as a function of work rate in CPET and SRT.

## Correlation between CPET and SRT exercise variables

Most peak exercise variables obtained during the SRT correlated excellently with those obtained during CPET (r .71 - .98; p <.01). Only the decrease in $SpO_2$% (r = .29; p = .33) and $RER_{peak}$ (*r* =.06; p = .85) were not significantly correlated between SRT and CPET (Table 3).

**Table 2.** Comparison of rest and peak exercise variables in the CPET and SRT

| | Variables | CPET | SRT | Difference | % | p | η2 |
|---|---|---|---|---|---|---|---|
| Rest | HR (beats·min$^{-1}$) (n=12) | 98.5±17.9 | 120.6±23.1 | +22.1 | +22.4 | .00* | .88 |
| | $VO_2$ (ml·kg$^{-1}$·min$^{-1}$) (n=13) | 7.2±1.8 | 7.5±2.1 | +0.3 | +4.2 | .53 | .03 |
| | RER ($VCO_2$·$VO_2^{-1}$) (n=13) | 0.91±0.06 | 0.85±0.04 | -0.06 | -6.6 | .00* | .53 |
| | VE (L·min$^{-1}$) (n=13) | 11.6±4.8 | 13.8±4.5 | +2.2 | 19.0 | .00* | .54 |
| | Oxygen saturation (%) (n=13) | 97.7±2.2 | 97.3±2.5 | -0.4 | 0.4 | .46 | .05 |
| Peak exercise | $VO_{2peak}$ (L·min$^{-1}$) (n=14) | 1.9±0.6 | 1.9±0.7 | 0 | 0 | .81 | .01 |
| | $VO_{2peak}$/kg (ml·kg$^{-1}$·min$^{-1}$) (n=14) | 38.9±7.4 | 38.8±8.5 | -0.1 | -0.3 | .81 | .00 |
| | $W_{peak}$ (Watt) (n=15) | 163.0±45.4 | 244.5±71.9 | 81.5 | +50.0 | .00* | .85 |
| | $W_{peak}$/kg (Watt·kg$^{-1}$) (n=15) | 3.3±0.5 | 4.9±0.8 | 1.6 | +48.5 | .00* | .90 |
| | $HR_{peak}$ (beats·min$^{-1}$) (n=13) | 177.2±11.9 | 179.2±13.1 | 2.0 | +1.1 | .35 | .07 |
| | HRR (n=12) | 53.7±18.6 | 41.5±22.6 | -12.2 | -22.7 | .00* | .66 |
| | $RER_{peak}$ ($VCO_2$·$VO_2^{-1}$) (n=14) | 1.2±0.1 | 1.0±0.1 | -0.2 | -16.6 | .00* | .59 |
| | BR (%) (n=13) | 29.0±14.3 | 30.2±16.6 | +1.2 | +4.1 | .60 | .02 |
| | $VE_{peak}$ (L·min$^{-1}$) (n=14) | 69.5±25.2 | 70.6±31.6 | +1.1 | +1.5 | .66 | .02 |
| | Δ Oxygen saturation (n=13) | -2.4±2.5 | -2.5±1.5 | +0.1 | +4.2 | 0.83 | .00 |

Legend: Values are means ± SD; η2= Partial Eta squared**;** HR = heart rate; $VO_2$ = oxygen uptake; RER= respiratory exchange ratio; VE = minute ventilation; W = work rate; HRR = heart rate response; BR = breathing reserve (%); Δ Oxygen saturation = Resting oxygen saturation – Oxygen saturation at peak exercise; *p <.01

## Agreement between the measured absolute and relative $VO_{2peak}$ in CPET and SRT

No systemic bias was noted for CPET and SRT measurements of $VO_{2peak}$/kg values. (Figure 2) The mean differences between CPET and SRT were 0.2 $L \cdot min^{-1}$ and 0.2 $ml \cdot min^{-1} \cdot kg^{-1}$ for absolute $VO_{2peak}$ and $VO_{2peak}$/kg values respectively. Limits of agreement between CPET and SRT $VO_{2peak}$/kg were -5.1 to 5.4 ml/kg/min.

There were only fair degrees of association of CPET and SRT differences in $VO_{2peak}$ and $VO_{2peak}$/kg with CPET and SRT mean $VO_{2peak}$ and $VO_{2peak}$/kg [r = -.37; p = .20 and r = -.42; p = .14 respectively].

**Table 3.** Pearson correlation coefficient between the CPET and SRT measurements

| Variables | Pearson *r* |
|---|---|
| $VO_{2peak}$ CPET – $VO_{2peak}$ SRT ($L \cdot min^{-1}$) | .98* |
| $VO_{2\,peak}$/kg CPET – $VO_{2\,peak}$/kg SRT ($ml \cdot min^{-1} \cdot kg^{-1}$) | .95* |
| $W_{peak}$ CPET – $W_{peak}$ SRT | .91* |
| $W_{peak}$/kg CPET – $W_{peak}$/kg SRT | .71* |
| $HR_{peak}$ CPET – $HR_{peak}$ SRT | .82* |
| RER CPET – RER SRT | .06 |
| $VE_{peak}$ CPET – $VE_{peak}$ SRT | .97* |
| BR CPET – BR SRT | .87* |
| Decrease in $SpO_2$% CPET – Decrease in $SpO_2$% SRT | .29 |

Abbreviations: $VO_{2peak}$ = peak oxygen uptake; $W_{peak}$ = peak work rate; $HR_{peak}$ = peak heart rate; RER= respiratory exchange ratio; $VE_{peak}$ = peak minute ventilation; BR = breathing reserve (%); *P<0.01; *r* = Pearson correlation coefficient.

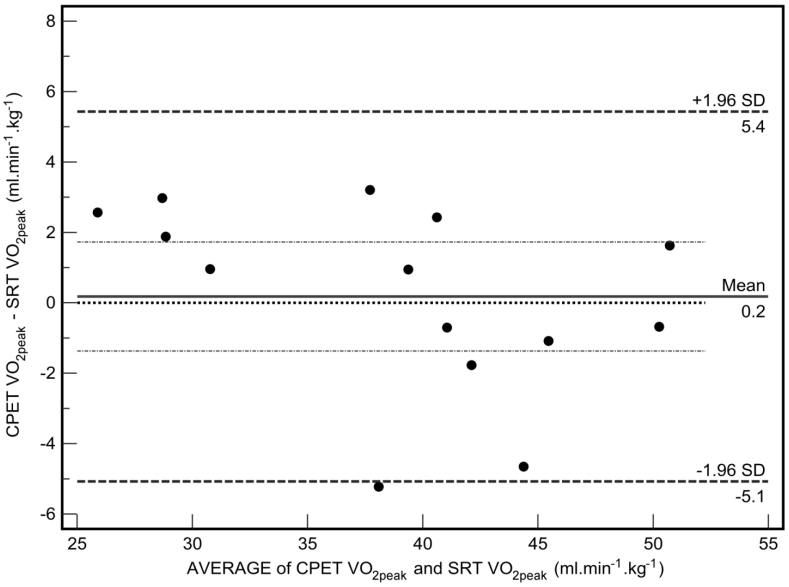


**Fig. 2.** Bland-Altman plot of the $VO_{2peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$) attained during CPET and SRT showing the bias and limits of agreement.

## Maximal effort criteria

All participants showed all the signs of subjective criteria. Based on the objective criteria, 7 participants performed a maximal effort and 8 did not. CPET $HR_{peak}$ missed in one person, so this persons' performance could not be classified according the objective criteria. Individual data are presented in Table 4.

No differences were found between CPET and SRT $VO_{2peak}$ values within the maximal and non-maximal effort group ($p = .85$ for $VO_{2peak}$ and $p = .54$ for $VO_{2peak}$/kg in the non-maximal effort group and $p = .40$ for $VO_{2peak}$ and $p = .63$ for $VO_{2peak}$/kg in the maximal effort group). Furthermore, no differences were found in CPET $VO_{2peak}$ values and SRT $VO_{2peak}$ values between the maximal and non-maximal effort group ($p = .62$ for CPET $VO_{2peak}$ and $p = .46$ for CPET $VO_{2peak}$/kg; $p = .98$ for SRT $VO_{2peak}$ and $p = .86$ for SRT $VO_{2peak}$/kg). Data *grouped by effort* are presented in Table 5.

**Table 4.** Individual data on maximal effort criteria

| Participant | $HR_{peak}$ (bpm) | $RER_{peak}$ | $VO_{2peak}$/kg-$VO_{2peak}$/kg last minute ($mL \cdot min^{-1} \cdot kg^{-1}$) | No. of Rowland Criteria Met (of 3) | $VO_{2peak}$/kgSRT -$VO_{2peak}$/kgCPET ($mL \cdot min^{-1} \cdot kg^{-1}$) |
|---|---|---|---|---|---|
| 1 | 174[a] | 1.03 | -0.02 | 2[b] | - 1.88 |
| 2 | 180[a] | 1.16 | 1.6 | 2[b] | - 0.68 |
| 3 | 183[a] | 1.27 | 3.1[a] | 1 | + 3.21 |
| 4 | 175[a] | 1.29 | 3.2 [a] | 1 | - 1.77 |
| 5 | 186 | 1.18 | 2.6 [a] | 2[b] | m.v. |
| 6 | 165[a] | 1.13 | 2.4 [a] | 1 | - 5.22 |
| 7 | 196 | 1.22 | 2.1 | 3[b] | - 1.08 |
| 8 | 180[a] | 1.15 | 2.6[a] | 1 | + 1.63 |
| 9 | 183[a] | 1.14 | 4.0[a] | 1 | + 2.43 |
| 10 | m.v. | 1.21 | 3.4[a] | m.v. | - 4.65 |
| 11 | 180[a] | 1.23 | 1.7 | 2[b] | + 2.57 |
| 12 | 182[a] | 1.14 | 6.4[a] | 1 | + 0.95 |
| 13 | 155[a] | 1.17 | 2.7[a] | 1 | + 2.98 |
| 14 | 183[a] | 1.31 | 0.4 | 2[b] | m.v. |
| 15 | 192 | 1.12 | 2.7[a] | 2[b] | - 0.7 |
| 16 | 158[a] | 0.97[a] | 4.3[a] | 0 | + 0.96 |

Legend: a Did not meet the maximal effort criterion, b Maximal effort according to criteria; abbreviations see legend to Table 2.

**Table 5.** Data grouped by effort

| | No Maximal effort | Maximal effort |
|---|---|---|
| $VO_{2peak}$CPET ($L \cdot min^{-1}$) | 2.0 ± 0.7*** (n=8) | 2.0 ± 0.6*** (n = 7) |
| $VO_{2peak}$SRT ($L \cdot min^{-1}$) | 2.0 ± 0.8*** (n = 8) | 2.0 ± 0.7*** (n = 5) |
| $VO_{2peak}$/kgCPET ($mL \cdot min^{-1} \cdot kg^{-1}$) | 38.8 ± 6.8*** (n = 8) | 38.5 ± 9.8*** (n = 7) |
| $VO_{2peak}$/kgSRT ($mL \cdot min^{-1} \cdot kg^{-1}$) | 38.2 ± 7.1*** (n = 8) | 38.1 ± 11.4*** (n = 5) |

Legend: * No significant difference between CPET and SRT grouped by effort, ** No significant difference in CPET in effort group and SRT in effort group, *** No differences between effort group and within effort group; abbreviations see legend to Table 2.

## DISCUSSION

The objective of this investigation was to verify the $VO_{2peak}$ attained during CPET in adolescents with CF using a supra-maximal exercise test (the SRT). We found no significant difference in $VO_{2peak}$ between CPET and SRT overall and when grouped on maximal effort. Our study indicates that the $VO_{2peak}$ attained during CPET reflects the true $VO_{2peak}$ in adolescents with mild-to-moderate CF even when the criteria of maximal effort were not met.

This study extends previously reported findings in healthy children and adolescents,[5, 7, 13, 14, 25] and ambulatory children and adolescents with spina bifida.[8]

In addition, the cardio respiratory demand of the SRT was comparable with CPET, as reflected by similar $VE_{peak}$, $HR_{peak}$ and $BR_{peak}$ values. HRR was even lower in the SRT, although this can be explained by the observed higher resting HR at the start of the SRT. Although $W_{peak}$ was significantly higher (~50%) in the SRT, and the SRT $VO_{2peak} \cdot kg^{-1}$ was comparable (100.3 ± 8% of the $VO_{2peak} \cdot kg^{-1}$) to that obtained during CPET, as were other peak cardiorespiratory parameters. This indicates that our supra-maximal test was more rigorous than previously used protocols using 105% to 110% of peak work rate attained during CPET,[7, 14] but gave comparable results for $VO_{2peak} \cdot kg^{-1}$. Furthermore, oxygen consumption for comparable workloads seems to be less during the SRT, which can be explained by a larger portion of anaerobic metabolism in energy supply. However, to our knowledge, the validity of the SRT as a measure of exercise capacity and the validity of the SRT as test of verification of attained $VO_{2peak}$ during CPET has not been studied yet.

In addition, the drop in $SpO_2$% was comparable during the CPET and the SRT, indicating that exercise-induced hypoxemia was comparable between the 2 protocols. However, we

used a finger sensor which might be less sensitive comparable to a forehead sensor to detect a drop in $SpO_2$%.[20] On the other hand, the drop in $SpO_2$% will be of a very short duration during the SRT. The incremental exercise phase lasted only 2 minutes during the SRT, and the effects of oxygen desaturation will be minimal. Additionally, $SpO_2$% was monitored during recovery and only one patients $SpO_2$% (88%) did not recover to <90% within one minute. However, the validity of the SRT in adolescents with CF and severe arterial hypoxemia and ventricular arrhythmias is unknown and future work is needed.

Compared to known reference values obtained in healthy Dutch adolescents, $VO_{2peak}$ and $W_{peak}$ values during CPET were decreased (87% and 72% of predicted respectively) in adolescents with CF.[26] This is in agreement with previous literature. Compared to adolescents with similar degrees of pulmonary dysfunction, $VO_{2peak}$ and $W_{peak}$ were higher in this study.[28-30] Comparable values were found for $HR_{peak}$.[30] This various reported limited exercise capacity in adolescents with cystic fibrosis (CF) is suggested to have multi-factorial cause. It seems that there is an interrelationship between lung function, muscle mass, energy expenditure, (respiratory) muscle function and exercise capacity in patients with CF.[31]

In the literature, several maximal criteria for CPET are suggested, but there is no agreement on how many criteria should be used, or the proportion that needs to be satisfied to confirm the validity of the $VO_{2max}$ test results.[32] For instance, Rowland suggests HR, RER and $VO_{2peak}$ plateau criteria during cycle ergometry as good indicators of maximal effort in pediatric exercise testing.[4] The current study suggests that these guidelines from healthy participants might not always be valid for clinical paediatric populations. Our lower $HR_{peak}$ data in adolescents with CF are comparable with other studies,[9-13, 33] and suggest that patients with CF have a lower $HR_{peak}$.

## Limitations and Future Research

This study was performed in adolescents with mild-to-moderate CF ($FEV_1$%predicted [range 45-117%]). The validity of the SRT as a measure of exercise capacity and the validity of the SRT as test of verification of attained $VO_{2peak}$ during CPET remains to be determined in more severe patients with CF, as well as in younger patients with CF. Furthermore, future work considering the validity of the SRT in adolescents with CF and severe arterial hypoxemia and ventricular arrhythmias is needed.

Before the SRT, we found higher resting HR and VE, accompanied by a lower RER ($p < .01$), pointing to incomplete recovery after CPET, so comparisons between resting values should be made with caution. Furthermore, a partially recovered metabolism could possibly result in a faster onset of oxygen uptake kinetics at the start of the SRT,[34] leading to a higher SRT $VO_{2peak}$. On the other hand, as the metabolism was partially recovered and as peripheral muscle fatigue was the primary reason for ending the test, it is possible that

the effect of fatigue before the SRT has influenced peak exercise parameters in this test. Nonetheless, no difference was noted in resting $VO_2$, indicating (nearly) full metabolic recovery in the exercise parameter of interest before the SRT. In order to correct for the effect of test sequence, at present, we are studying the validity of the SRT to measure $VO_{2peak}$ with counterbalanced test sequence.

## Conclusion

As verified with a supra-maximal exercise test, the $VO_{2peak}$ measured during CPET seems to reflect the true $VO_{2peak}$ in adolescents with CF. The SRT seems to be an appropriate and well-tolerated protocol for the supra-maximal verification of $VO_{2peak}$ in adolescents with mild-to-moderate CF.

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Chapter
2

# Chapter 3

# Towards an individualized protocol for workload increments in cardiopulmonary exercise testing in children and adolescents with cystic fibrosis

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## ABSTRACT

*Background*: There is no single optimal exercise testing protocol for children and adolescents with cystic fibrosis (CF) that differ widely in age and disease status. The aim of this study was to develop a CF-specific, individualized approach to determine workload increments for a cycle ergometry testing protocol.

*Methods*: A total of 409 assessments consisting of maximal exercise data, anthropometric parameters, and lung function measures from 160 children and adolescents with CF were examined. 90% of the database was analyzed with backward linear regression with peak workload ($W_{peak}$) as the dependent variable. Afterwards, we [1] used the remaining 10% of the database (model validation group) to validate the model's capacity to predict $W_{peak}$ and [2] validated the protocol's ability to provide a maximal effort within a 10±2 minutes time frame in 14 adolescents with CF who were tested using this new protocol (protocol validation group).

*Results*: No significant differences were seen in $W_{peak}$ and predicted $W_{peak}$ in the model validation group or in the protocol validation group. Eight of 14 adolescents with CF in the protocol validation group performed a maximal effort, seven of them terminated the test within the 10±2 minutes time frame. Backward linear regression analysis resulted in the following equation: $W_{peak}$ *(Watt) = -142.865 + 2.998 x Age (years) – 19.206 x Sex (0 = male; 1 = female) + 1.328 x Height (cm) + 23.362 x* $FEV_1$ *(L)* (R=.89; $R^2$ = .79; SEE = 21). Bland-Altman analysis showed no systematic bias between the actual and predicted $W_{peak}$.

*Conclusion*: We developed a CF-specific linear regression model to predict peak workload based on standard measures of anthropometry and $FEV_1$, which could be used to calculate individualized workload increments for a cycle ergometry testing protocol.

## INTRODUCTION

The clinical utility of exercise and exercise testing in the diagnosis and treatment as well as the primary and secondary prevention of chronic conditions is widely recognized by health care professionals. This is evidenced by the fact that cardiopulmonary exercise testing (CPET) is increasingly used to evaluate exercise capacity and define training intensity in adolescents with chronic lung diseases like cystic fibrosis (CF).[1,2] Numerous protocols have been used in the past to evaluate physical fitness in adolescents with chronic conditions, but there is still no consensus with regards to the best protocol to be used in the clinical setting. Moreover, there is some question as to whether these exercise testing protocols should vary by pediatric condition or even between patients with the same condition. Ultimately, the characteristics of the patient and the purpose of the exercise test will determine which protocol is most suited for each individual patient. Aside from patient characteristics, it has been suggested that the length of the exercise test represents an additional important criterion to consider when designing a protocol that will elicit maximal or peak oxygen uptake and demonstrate good reproducibility of commonly measured exercise parameters (e.g. ventilatory threshold). While a test duration of 10 ± 2 minutes has been recommended to allow the patient to reach their limit of tolerance.[3,4] Midgley et al nuanced this recommendation by suggesting that cycle ergometer tests should last between 7 and 26 minutes with a focus on tolerable workload increments rather than test duration, per se.[5]

Progressive incremental cycle ergometer protocols, continuous incremental (ramp) protocols, and protocols with short stage duration (e.g. 1 minute) are very efficient at inducing maximal exercise responses in an optimal time frame (~ 10 to 12 minutes). The Godfrey protocol was the first 1-minute incremental protocol systematically used in pediatric CF patients.[6,7] This protocol separates subjects into 3 groups based on height with workload increments increasing with height from 10 watt to 15 watt and 20 Watts per minute. The large differences in disease severity and the progressive nature of CF make a more individualized approach to the incremental exercise test critical. It is generally accepted that, to date, there is no single best exercise testing protocol to answer all questions in individuals with CF with a wide range of ages and disease states. [8,9] Therefore, we aimed to develop a CF-specific, individualized approach from standard measures of anthropometry and lung function in children and adolescents to determine workload increments for a maximal cycle exercise test.

## METHODS

Maximal effort cycle ergometer CPET data (n=409) from 160 children and adolescents with CF receiving care at the Cystic Fibrosis Center of the Wilhelmina Children's Hospital, Utrecht, the Netherlands, were analysed. Body weight, height, lung function and exercise capacity were determined as part of routine measures at the patient's annual medical check-up. Since all measurements were a part of patient standard of care, ethical approval and informed consent were not required according to Dutch Law.
Out of the database, a regression model was developed out of 90% of the data (reference group). Afterwards, we [1] used the remaining 10% of the database (model validation group) to validate the model's capacity to predict $W_{peak}$ and [2] validated the protocol's (minute increment = predicted $W_{peak}$ / 10) ability to provide a maximal effort within a 10±2 minutes time frame in 14 adolescents with CF who were tested using this new protocol (protocol validation group). The CPET was part of their usual care at the annual CF check-up.

### Spirometry

Spirometry and body plethysmography were performed before and after bronchodilation with salbutamol (800 ug), using a pneumotach apparatus and a volume-constant plethysmograph (Master Lab system, E. Jaeger, Würzburg, Germany). Lung function measurements included total lung capacity (TLC), residual volume (RV), and forced expiratory volume in 1 second ($FEV_1$). These results were compared with predicted values for healthy subjects matched for age, height, and gender.[10]

### Cardiopulmonary exercise test (CPET)

After brochodilation with salbutamol, all participants performed a progressive cardiopulmonary exercise test (CPET) on an electronically braked cycle ergometer (Jaeger physis; Carefusion, Houten, The Netherlands) to assess exercise capacity. Ergometer seat height was adjusted to the participant's comfort and leg length. Participants rested until all measured variables were stable, and then began cycling at a workload of 0 W; the workload was increased 15 W/min until the participant stopped due to volitional exhaustion. Throughout the exercise, participants breathed through a mask (Hans Rudolph Inc.,
Kansas City, MO) that was connected to a calibrated metabolic cart (Oxycon pro, Carefusion, Houten, The Netherlands). Expired gas was passed through a flow meter, oxygen analyzer, and carbon dioxide analyser, which were connected to a computer that calculated breath-by-breath minute ventilation (VE), oxygen uptake ($VO_2$), carbon dioxide production

($VCO_2$) and respiratory exchange ratio (RER) from conventional equations. Heart rate was monitored continuously by 3-lead electrocardiography (Hewlett-Packard, Amstelveen, Netherlands). The oxygen uptake and workload completed in the final 30 seconds prior to exhaustion were considered to be $VO_{2peak}$ and $W_{peak}$, respectively. Relative peak oxygen uptake ($VO_{2peak}$/kg) was calculated by dividing $VO_{2peak}$ by total body mass. All participants included in the analysis were required to have performed a maximal effort test, which was defined by a combination of subjective and objective criteria. Subjective criteria included subject exhaustion (unsteady biking, sweating, facial flushing) or an inability to maintain the required cadence of 60 revolutions per minute despite strong verbal encouragement. Objective criteria included: (1) peak heart rate ($HR_{peak}$) > 180 bpm [11] and (2) respiratory exchange ratio (RER) > 1.00. Based on previous literature, a true maximal effort required participants to demonstrate both objective and subjective criteria.[12]



### Statistics

All variables were tested for normality with the Kolmogorov-Smirnov test. Backward linear regression analysis was performed using the data of the reference group. Peak workload ($W_{peak}$) was defined as the dependent variable in the model, while gender, height, and $FEV_1$ (L) were selected as independent variables. The model validation group was used to validate the models capacity to predict $W_{peak}$. Student's t-tests or Mann-Whitney test were used to assess differences between the reference and validation groups for gender distribution, height, weight, $FEV_{1\%pred}$, $W_{peak}$ and $VO_{2peak}$. For the model validation group, a Bland-Altman plot was used to assess any systematic bias between actual $W_{peak}$ and $W_{peak}$ predicted. After developing the regression equation, we validated the new protocol in the protocol validation group.
Statistical significance was set at $p < 0.05$. All statistical analyses were performed in SPSS 15.0 for Windows (Chicago, IL, USA).

## RESULTS

Kolmogorov-Smirnov tests revealed that $FEV_1$ (L), $W_{peak}$, $HR_{peak}$, $RER_{peak}$ and time to exhaustion (Tlim) were not normally distributed. However, the residuals of the model predicting $W_{peak}$ were normally distributed. No complications were observed during or after exercise testing.
$RER_{peak}$ of the total group was 1.19±0.09 [range 1.0 – 1.67] and $HR_{peak}$ 190±7 [range 180 - 210] beats per minute (bpm).No differences in maximal exercise parameters were found between both groups (reference group $RER_{peak}$ 1.20±0.09 [range 1.0-1.67] and $HR_{peak}$ 189±7 [range 180-210 bpm] versus model validation group $RER_{peak}$ 1.18±0.09 [range 1.0 – 1.45]

and $HR_{peak}$ 190±6 [range 180-210 bpm]; p = .33 for $RER_{peak}$ and $HR_{peak}$).
Anthropometric characteristics of both groups are presented in Table 1. Mean $FEV_{1\%pred}$ for the validation and reference groups were 86.9±19.3% and 87.2±17.4% (p= .91), respectively. Gender distribution between the reference and validation group was similar (38% ♀ and 62% ♂ in the reference group vs. 45% ♀ and 55% ♂ in the validation group; $X^2$ test p = .503). No differences in age were seen between the validation and regression group (14.2±1.9 [11-18] vs. 14.5±2.0 [8-18] years; p= .41).

**Table 1.** Anthropometric values.

| | Age | Gender | Weight | Height | $FEV_{1pred}$ |
|---|---|---|---|---|---|
| Reference group | 14.5±2.0 | 17♀ 21♂ | 50.0±12.2 | 162.6±12.0 | 87.2±17.4 |
| Validation group | 14.2±1.9 | 140♀ 231♂ | 49.1±9.9 | 161.9±11.1 | 86.9±19.3 |

For the reference group, mean $W_{peak}$ was 171.2 ± 46.3 Watts with a mean Tlim of 11.4 minutes [range 5.0-20.0 minutes]. For the model validation group, mean $W_{peak}$ was 164.6±38.6 W, and mean Tlim was 11.0 minutes [range 7.0-16.0 minutes]. No differences were noted for $W_{peak}$ (p = .496) or Tlim (p = .496) between the reference and model validation groups.
Height, weight, $FEV_1$ (L and %predicted), gender, and age were all significantly associated with $W_{peak}$ (.319 - .803; p < .001) in pediatric patients with CF, with the strongest correlation found for $FEV_1$ (L) (*r* .803; p < .001) (see Table 2).
Inclusion of these variables in backward linear regression analysis resulted in the following equation:

Extrapolating this equation to the model validation group resulted in a predicted mean $W_{peak}$ of 164.6 ± 38.6 watt, compared with the actual mean $W_{peak}$ of 166.5±38.1 (p = .09). Bland-Altman analysis showed no systematic bias between the actual and predicted $W_{peak}$. (see Figure 1)

**Table 2.** Correlation coefficients for weight, age and lung function with Wpeak in males and females.

| | | $W_{peak}$ |
|---|---|---|
| Pearson Correlation | Age | .64 |
| | Sex | -.42 |
| | Weight | .78 |
| | $FEV_{1pp}$ | .32 |
| | Height | .80 |

Based on these findings, we conclude that the model developed in the present study to predict $W_{peak}$ from standard anthropometric variables is valid. Ultimately, this approach will provide more optimal CF-specific exercise testing of comparable test duration across a wide variety of ages and disease states.

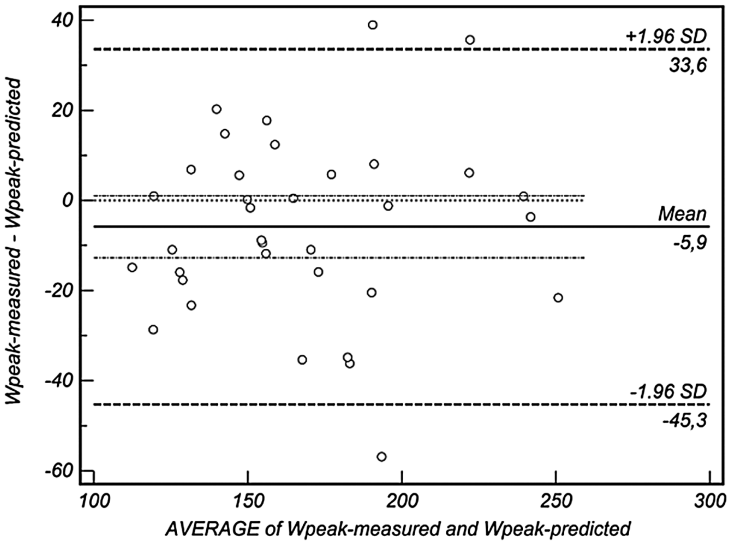


**Fig.1. Bland-Altman** plot of the measured Wpeak and predicted Wpeak in the model validation group showing the bias and limits of agreement. *Bland-Altman plot has been developed from the 10% validation group.

We validated the developed protocol (increments per minute = predicted $W_{peak}$ / 10) in 14 adolescents with CF. Six of them were excluded from further analysis while they did not meet the above mentioned criteria for maximal effort. The remaining eight participants (3 female, 5 male; age 15.6±2.2 years [12-19]; $FEV_1$ 3.0±0.8 L, $FEV_{1\%pred}$ 87±19 %; height 167±10 cm; weight 52±9 kg) were used to validate the protocol (Tlim 10.8±1.4 minutes [9.2 – 13.3 minutes]; $RER_{peak}$ 1.28±0.06 [range 1.21-1.40] and $HR_{peak}$ 185±4 [range 181-189 bpm]). Seven of them performed a maximal effort within the targeted 10±2 minutes time frame.

When we compare the Tlim of the CF-specific protocol with the estimated Tlim of the Godfrey protocol ($W_{peak}$ / 20 watts per minute), much more variance in Tlim would have been found in the Godfrey protocol (95% CI for CF specific protocol 9.7-12.0 min versus 7.8-12.0 minutes). (see Figure 2)

We found no difference between measured $W_{peak}$ and estimated $W_{peak}$ (197±50 watt versus 186±38 watt; p = .22). Bland-Altman analysis showed no systemic bias between actual and predicted $W_{peak}$ (mean difference 10.9 watt; LOA + 55.7; - 33.9 watt). (see Figure 3)

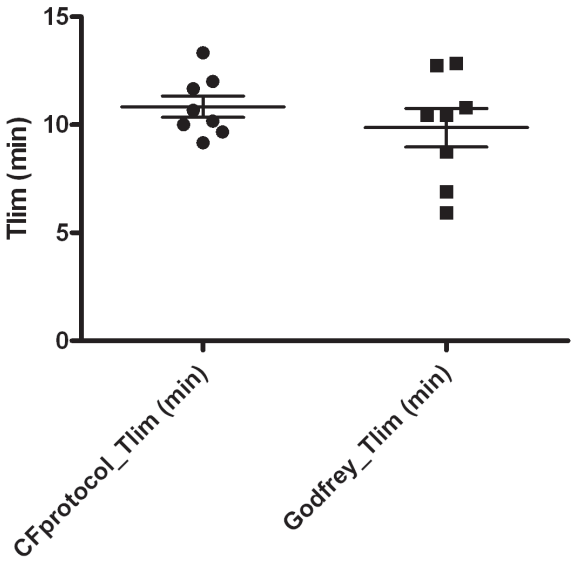


**Fig. 2.** Vertical point plot of the mean Tlim (95% CI) of both protocols.

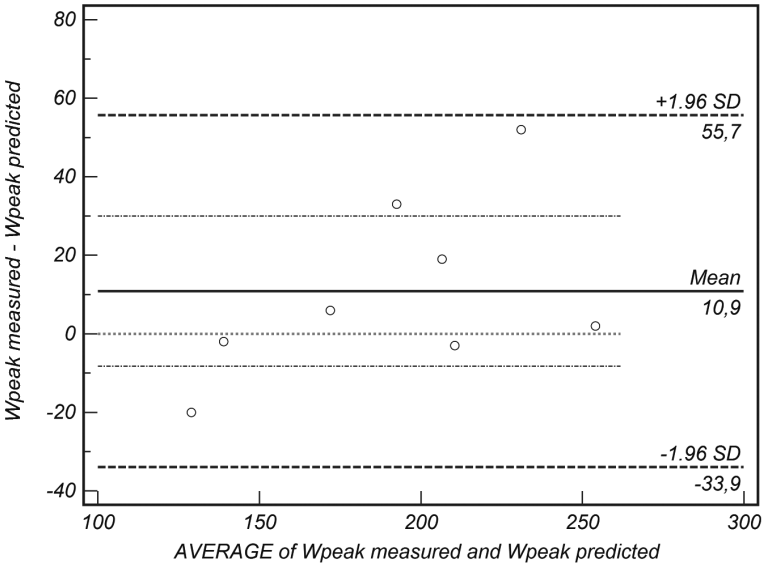


**Fig. 3.** Bland-Altman plot of the measured $W_{peak}$ and predicted $W_{peak}$ in the protocol validation group.

## DISCUSSION

We aimed to develop a CF-specific linear regression model for children and adolescents to predict peak workload based on standard measures of anthropometry and $FEV_1$, which could then be used to calculate individualized workload increments for an exercise protocol wherein participants achieve cardiopulmonary exhaustion in approximately 10 minutes. While an individualized exercise protocol has previously been shown to be feasible in children and adolescents with various diseases, it is important to note that in that study the individualization was based on peak oxygen uptake rather than peak workload.[13] In the current study, height, weight, $FEV_1$, gender and age were significantly associated with $W_{peak}$ in adolescent patients with CF. Given the strength of the relationship between $W_{peak}$ and height, it seems that the exercise protocol developed by Godfrey, who based the increments on the individual's height (10 W/min < 120 cm; 15 W/min 120 – 150 cm; 20 W/min > 150 cm), already provides better individualization of the exercise increments for children and adolescents (with CF) than standard increments.



Generally, a workload increment that brings the subject to their limit of tolerance in about 10 min is suggested to provide optimal cardiopulmonary assessment.[4] Despite the grading of the workload increments according to size in the Godfrey protocol, the smallest healthy children and adolescents complete the test in about four minutes, whereas tests for the largest healthy children last roughly 10 minutes.[7] The preliminary termination seen in small children and adolescents may be due to workload increments that are too large, resulting in premature exhaustion of the muscles of the lower limbs before the attainment of cardiac or respiratory limits.[13] Additionally, $VO_2$ on-kinetics are reportedly slowed in steeper ramp slopes,[14] which may compromise the aerobic contribution to total energy delivery [14] and lead to premature exhaustion of the anaerobic energy system in the local muscles. Therefore, at least in smaller children and adolescents, as is the case in CF, a protocol based solely on a single variable such as height does not seem to provide the appropriate workload increments to achieve $VO_{2peak}$ in the suggested 10-minute time frame.

Conversely, a multivariable-based exercise protocol may allow for better selection of workload increments leading to more optimal exercise duration. Linear regression analysis showed gender, $FEV_1$, age and height to be significant and independent predictors of $W_{peak}$. The resulting model explained 79% of the variance in $W_{peak}$. Validation of the models capacity to predict $W_{peak}$ showed no significant difference between the measured $W_{peak}$ and estimated $W_{peak}$ in a sample of children and adolescents with CF. Moreover, Bland-Altman analysis showed no systematic bias between the measured $W_{peak}$ and estimated $W_{peak}$. Additionally, we validated the new incremental protocol and found no difference between measured $W_{peak}$ and estimated $W_{peak}$ (197±50 watt versus 186±38 watt; p = .22),

whereas the maximal effort was performed within the targeted 10±2 minute time frame in seven of the eight participants who performed a maximal effort.

To ensure a maximal effort, we used previously used modified criteria in our laboratory.[12] The achievement of these criteria was of critical importance as questions about cardiopulmonary limiting factors or cardiopulmonary exercise capacity can only be answered when the cardiopulmonary system has truly reached its limitations. Individualization of the exercise protocol has been shown to provide achievement of the maximal exercise criteria in approximately 10 minutes exercise duration in a higher proportion of children and adolescents.[13]

Based on these findings, we conclude that the model developed in the present study to predict $W_{peak}$ from standard anthropometric variables is valid. Dividing the predicted $W_{peak}$ by 10 to determine individualized workload increments for the maximal exercise test will allow participants to achieve true cardiopulmonary exhaustion in the recommended 10-minute time frame. Ultimately, this approach will provide more optimal CF-specific exercise testing of comparable test duration across a wide variety of ages and disease states. However, the equation has not been tested or validated in adult patients with CF. Further research to cross-validate this individualized protocol in adolescents and adults with CF is suggested. Additionally, CF-specific normative data for $W_{peak}$ are required for the interpretation of the test results.

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# Chapter 4

# Estimating peak oxygen uptake in adolescents with cystic fibrosis

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## ABSTRACT

*Background*: To predict peak oxygen uptake ($VO_{2peak}$) from the peak work rate ($W_{peak}$) obtained during a cycle ergometry test using the Godfrey protocol in adolescents with CF and assess the accuracy of the model for prognostication clustering.
*Methods*: Out of our database of anthropometric, spirometric and maximal exercise data from adolescents with CF (N = 363; 140 girls and 223 boys; age 14.77±1.73 years; mean expiratory volume in 1 second ($FEV_{1\%pred}$) 86.82±17.77 %), a regression equation was developed to predict $VO_{2peak}$ ($mL{\cdot}min^{-1}$). Afterwards, this prediction model was validated with cardiopulmonary exercise data from another sixty adolescents with CF (28 girls, 32 boys; mean age 14.6 ±1.67 years; mean $FEV_{1\%pred}$ 85.43±20.01%).
*Results*: We developed a regression model $VO_{2peak}\ [mL{\cdot}min^{-1}] = 216.3 - 138.7 \times Sex$ (0=male; 1=female) + $11.5 \times W_{peak}$ ; $R^2$= .91; standard error of the estimate (SEE) 172.57. A statistically significant difference (107 $mL{\cdot}min^{-1}$; $p < .001$) was found between predicted $VO_{2peak}$ and measured $VO_{2peak}$ in the validation group. However, this difference was not clinical relevant because the difference was within SEE of the model. Furthermore, we found high positive predictive and negative predictive values for the model for prognostication clustering (PPV 50-87% versus NPV 82-94%).
*Conclusions*: In the absence of direct $VO_{2peak}$ assessment it is possible to estimate $VO_{2peak}$ in adolescents with CF using only a cycle ergometer. Furthermore, the regression model showed to be able to discriminate patients in different prognosis clusters based on exercise capacity.

## INTRODUCTION

Cystic Fibrosis (CF) is the most common lethal autosomal recessive childhood disorder in the white population, occurring in approximately 1 in 2500 births. The disease is caused by a defect of the cystic fibrosis transmembrance conductance regulator (CFTR) gene, which causes clinical manifestations in multiple organ systems as the lungs, intestines and pancreas.[1]

Low exercise capacity has been reported in children and adolescents with CF which seems to have a multi-factorial cause.[2] Furthermore, significant associations have been reported between exercise capacity of patients with Cystic Fibrosis (CF) and survival over an 8-10 years period.[3, 4] The most important parameter of aerobic exercise capacity is peak oxygen uptake ($VO_{2peak}$),[5-8] commonly defined as the highest oxygen uptake attained during a single progressive cardiopulmonary exercise test (CPET).[9] A CPET plays an important role in cystic fibrosis (CF) care and follow-up because of its contributing diagnostic, prognostic and functional information.[5] As mentioned previously, $VO_{2peak}$ is a significant predictor of subsequent mortality, both as percentage of predicted [3, 10] or as absolute value $ml \cdot min \cdot kg^{-1}$.[4] Pianosi states that a $VO_{2peak} < 32\ ml \cdot min \cdot kg^{-1}$ was associated with a 10 year mortality of 50%, whereas a $VO_{2peak} > 45\ ml \cdot min \cdot kg^{-1}$ showed an association of 100% in 10 year survival.[4]

Despite its clinical and prognostic value, many specialized CF centers still do not perform exercise testing, with or without gas analysis. A recent survey in UK CF clinics indicated that availability of resources to directly measure $VO_{2peak}$ (metabolic gas analysis system with treadmill or cycle ergometer) was the main reason for this.[11] In centres without CPET possibilities, walking tests are frequently used as an alternative for $VO_{2peak}$ assessments because they offer a simple and inexpensive means of estimating exercise capacity.[11, 12] Recent evidence however suggests that these field tests are not very strongly associated with $VO_{2peak}$ in children and adolescents with CF.[13]

The Godfrey protocol [14] is a validated cycle protocol to measure $VO_{2peak}$ and is has been designed to induce exhaustion within 10 to 12 minutes and is frequently used in patients with CF.[15, 16] Using an incremental exercise test protocol, a strong relation between $VO_{2peak}$ and $W_{peak}$ has been reported (coefficients of determination ($R^2$) 0.98±0.03) in healthy children [17] and in adolescents with CF *(**r** = 0.91; p<0.001)*.[*18*]

This might implicate that, theoretically, CPET using the Godfrey protocol measuring only $W_{peak}$ (i.e. without gas analysis) could provide an alternative and valid method for the prediction of $VO_{2peak}$ in adolescents with CF. A valid and inexpensive exercise test may help to increase the utilization of exercise testing in the clinical care and research of this patient group.[11, 12, 19, 20] Furthermore, more thorough assessment of exercise capacity might have its impact on planning of lung transplantation as exercise testing is considered as an

important prognostic tool for the selection of pediatric lung-transplant candidates with end-stage CF.[12]
Therefore, in order to optimize the utility of clinical exercise testing, the objectives of this study were (1) to predict $VO_{2peak}$ without gas analyses from $W_{peak}$ on a cycle ergometer, using the Godfrey protocol in adolescents with CF and (2) assess the accuracy of this prediction model for prognostication clustering.

## METHODS

### Study subjects

Out of a database of anthropometric, spirometric and maximal exercise data from adolescents with CF (= reference group; N = 363) tested in our laboratory between 1996 and 2006, a regression equation was developed to predict $VO_{2peak}$ ($mL \cdot min^{-1}$).
Another sixty adolescents with CF (= validation group) also performed a CPET using the Godfrey protocol at their annual medical check-up. This group was used to validate the regression equation and to asses the accuracy of the model for prognostication clustering.
Since exercise testing is a part of standard medical care in our CF center, no medical-ethical approval or written informed consent was required according to the Dutch law for medical research. The medical ethical committee of the University Medical Centre Utrecht approved the use of the database with anonymous patient care data of patients with CF for scientific purposes.
Individual data were collected over the course of one visit. Adolescents were asked to avoid heavy meals and strenuous exercise as of the evening before their testing session. First, lung function (Master Lab system, E. Jaeger, Würzburg, Germany) and anthropometric values, including weight and height were measured using an electronic scale (Seca, Birmingham, UK) and a stadiometer (Ulmer stadiometer, Prof. E. Heinze, Ulm, Germany), respectively. This was followed by the performance of the CPET. We used the anthropometric, spirometric and exercise data of the patients in the database who performed a maximal effort ($HR_{peak}$ > 180 bpm [21], $RER_{peak}$ > 1.0 and subjective signs of voluntary exhaustion. For a maximal effort, participants had to meet all the criteria.[9]

### Godfrey exercise protocol

The Godfrey protocol was performed on an electronically braked cycle ergometer (Lode Corrival, Procare BV, Groningen, The Netherlands). Participants began with unloaded cycling and the workload increased every minute in a fixed interval based on height (10 W/min < 120 cm; 15 W/min 120 – 150 cm; 20 W/min > 150 cm), independent of

sex, until the patient stopped due to volitional exhaustion.[14] Throughout the test, adolescents breathed into a mouthpiece connected to a calibrated metabolic cart (ZAN 600, Accuramed Bv, Lummen, Belgium). Expired gas passed through a flow meter, oxygen analyzer, and a carbon dioxide analyzer. The flow meter and gas analyzer were connected to a computer, which calculated breath-by-breath minute ventilation (VE), oxygen uptake ($VO_2$), carbon dioxide production ($VCO_2$), and respiratory exchange ratio (RER) from conventional equations. Heart rate (HR) was also monitored continuously by a 12-lead electrocardiogram (Cardioperfect, Accuramed bv, Lummen, Belgium), and transcutaneous oxygen saturation ($SpO_2$%) was measured by a pulse oximeter placed on the index finger (Nellcor 565, Coviedien, Zaltbommel, The Netherlands). Peak exercise parameters were defined as the mean values achieved during the final 30 seconds of the test.

## Statistical analysis



Data were expressed as mean ± SD. Data were analyzed using SPSS PASW Statistics 17.0 for Windows (SPSS Inc, Chicago, Ill, USA) and tested for normality with the Kolmogorov-Smirnov Test. A p-value of $<0.05$ was considered statistically significant. A linear regression model (backwards-elimination procedure) from the data of the reference group was used to predict $VO_{2peak}$ ($mL \cdot min^{-1}$) based on the $W_{peak}$ combined with standard anthropometric variables based on biological plausibility (height (cm), age (years), sex (0=male; 1=female) and lung function ($FEV_1$ ($L \cdot min^{-1}$)). Variables were excluded from the regression when $p > 0.1$. Exercise data of the validation group were used to measure the accuracy of the model for prognostication clustering. Paired sample t-tests or Wilcoxon signed ranks tests were used to analyse possible differences between actual and predicted $VO_{2peak}$. A Bland-Altman plot was used to assess any systematic bias between measured $VO_{2peak}$ and predicted $VO_{2peak}$. Additionally, the same linear regression procedure as for the reference group was performed in the validation group to analyse for different variables being entered in the model.

Afterwards, the measured and predicted $VO_{2peak}$ of the participants in the validation group who performed a maximal effort were clustered in three prognostic groups based on high ($> 45$ $ml \cdot min \cdot kg^{-1}$), medium (32-45 $ml \cdot min \cdot kg^{-1}$) and low ($<32$ $ml \cdot min \cdot kg^{-1}$) $VO_{2peak}$ as previously described by Pianosi et al.[4]

## RESULTS

Out of a database of anthropometric, spirometric and maximal exercise data from adolescents with CF (= reference group) (N = 363, 140 girls and 223 boys, mean age 14.77±1.73 years, and mean $FEV_{1\%pred}$ 86.82±17.77 %) tested in our laboratory between 1996 and 2006.The characteristics of the reference group are presented in Table 1.

**Table 1.** Patient characteristics and peak exercise data

| | Reference group (n363) | Validation group (n=60) |
|---|---|---|
| Age (years) | 14.77±1.73 [12.08 – 18.33] | 14.58±1.67 |
| Weight (kg) | 51.31±11.39 [30.10 – 94.60] | 50.44±9.68 |
| Height (cm) | 164.20±10.75 [134.80 – 190.10] | 165.29±11.92 |
| Sex | 223♀ 140♂ | 28 girls; 32 boys |
| $FEV_1$%predicted ($FEV_1$ (L)) | 86.82±17.77 (2.72±0.82) [37-147] | 85.43±20.01 (2.71±.94) |
| $HR_{peak}$ (bpm) | 190±7 [180-210] | 180±12 |
| $RER_{peak}$ | 1.2±0.1 [1.0-1.7] | 1.13±0.11 |
| $W_{peak}$ (watt) | 174±45 [75-300] | 171±46 |
| $VO_{2peak}$ ($mL{\cdot}min^{-1}$) | 2151±571 [1000-3800] | 2019±567 |

*Prediction of the $VO_{2peak}$ from the $W_{peak}$*

Linear regression revealed the following equation (95% prediction interval between 1770 and 2548 $mL{\cdot}min^{-1}$), with $W_{peak}$ and sex as the only significant contributors (see Table 2).

$$VO_{2peak}\,(mL{\cdot}min^{-1}) = 216.3 - 138.7 \times Sex\ (0=female\ /\ 1=male) + 11.5 \times W_{peak}$$

**Table 2.** Final regression model

| ***Final regression model*** | **R** | **$R^2$** | **SEE** | **p-value** | **95% PI** |
|---|---|---|---|---|---|
| | .954 | .909 | 172.57 | <.001 | [1770-2548] |
| Outcome variable | Predictor variable | Unstandardized Beta | Standardized Beta | 95% CI | p-value |
| $VO_{2peak}$ ($mL{\cdot}min^{-1}$) | Constant | 216.342 | | 128.360 – 304.324 | <.001 |
| | Sex | -138.713 | -0.118 | -180.004 – -97.423 | <.001 |
| | $W_{peak}$ | 11.445 | 0.897 | 10.996 – 11.895 | <.001 |

The greatest contributor to this regression equation was $W_{peak}$ followed by sex. When all the variables were entered in the equation, Age (beta = -.02; p =.42), Height (beta = -.02; p = .41) and $FEV_1$ (beta = .03; p = .34) did not make a significant additional contribution.

## Cross-validation

All sixty participants in the validation group successfully performed CPET without complications or adverse events. Descriptive characteristics are presented in Table 1.
Based on previous mentioned criteria, 36 performed a maximal effort (20 ♀ 16 ♂, age 14.6±1.7 years, $FEV_{1\%}$ 86.89±18.67 %, $HR_{peak}$ 188±7 bpm, $RER_{peak}$ 1.16±0.08). Their data were used to calculate the differences between measured and predicted $VO_{2peak}$.
We found a small but statistically significant difference (mean difference 107 $mL \cdot min^{-1}$; $p < .01$) between predicted $VO_{2peak}$ (2231±550 $mL \cdot min^{-1}$) and measured $VO_{2peak}$ (2125±544 $mL \cdot min^{-1}$). However, Bland-Altman analysis and an XY plot showed no systemic bias, with acceptable limits of agreement (see Figure 1 and 2).
Furthermore, linear regression revealed the following equation for the validation group, with $W_{peak}$ (Standardized beta .83; $p < .001$) and sex (Standardized beta -.17; p .038) as the only significant contributors:

$$VO_{2peak}\,(mL \cdot min^{-1}) = 377.0 - 178.4 \times Sex\ (0=female\ /\ 1=male) + 10.1 \times W_{peak}$$
$$R = .921;\ R^2 = .848;\ SEE = 218.48;\ p < .001$$

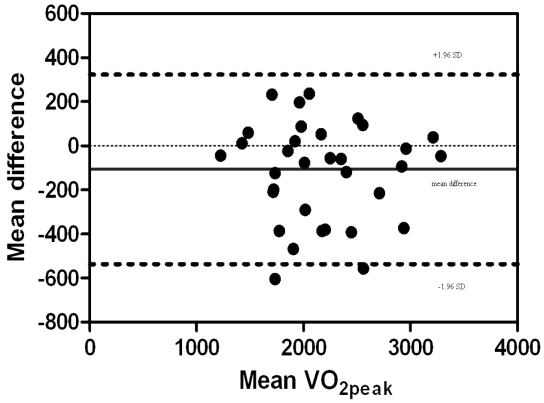


**Fig.1.** Bland-Altman plot of the predicted and measured $VO_{2peak}$ in the validation group.

## Prognostics

The positive predictive value for the model to correctly assign patients to the low, medium or high $VO_{2peak}$ prognosis group were 87%, 74% and 50% respectively. The negative predictive value for the model to correctly assign patients as not having a low, medium or high $VO_{2peak}$ were 86%, 82% and 94% respectively (see Table 3).

**Table 3.** Prognostication based on measured versus predicted VO2peak

| | | Prognosis using measurement | | | |
|---|---|---|---|---|---|
| | | Low | Medium | High | *Total* |
| Prognosis using model | Low | 1 | 1 | 0 | 2 |
| | Medium | 2 | 14 | 3 | 19 |
| | High | 0 | 2 | 13 | 15 |
| | *Total* | 3 | 17 | 16 | 36 |

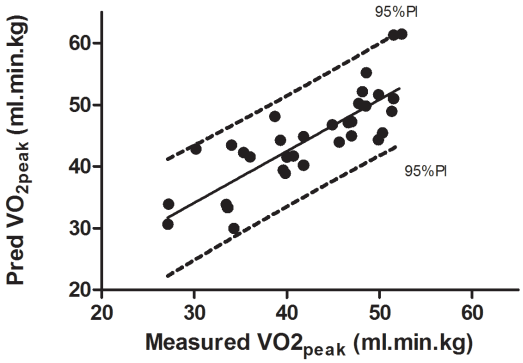


**Fig.2.** Scatter plot of the predicted and measured $VO_{2peak}$ in the validation group.

## DISCUSSION

The objectives of this study were (1) to predict $VO_{2peak}$ from $W_{peak}$ on a cycle ergometer using the Godfrey protocol in adolescents with CF and (2) assess the accuracy of the model for prognostication clustering.

We found a strong ($R^2$ =.91; SEE = 172.57) prediction model to predict $VO_{2peak}$ ($mL{\cdot}min^{-1}$) out of $W_{peak}$ and sex in a group of adolescents with CF with a large range in pulmonary function ($FEV_{1\%pred}$ [37 – 147 %]) with a 95% prediction interval between 1770 till 2548 $mL{\cdot}min^{-1}$. This result is in line with a previous study, which reported a strong relation between $VO_{2peak}$ and $W_{peak}$ (coefficients of determination ($R^2$) 0.98±0.03) in healthy children [*17*] and in children with CF *(***r** *= 0.91; p<0.001).*[*18*] However, the slope of the $VO_2$ as response to the workrate increment ($\Delta O_2/\Delta W$) was higher in children with CF compared to healthy controls.[*17*] This could suggest a high oxygen consumption of the respiratory muscles by a higher work of breathing in patients with lung disease.[*22, 23*] In patients with CF, especially in a more severe disease status, several mechanisms become involved such as an increased work of breathing during exercise.[*2*]

Although we observed statistically significant differences between the predicted $VO_{2peak}$ and the measured $VO_{2peak}$ in the validation group ($p < .01$), this difference (107 $mL \cdot min^{-1}$) was quite small and within the SEE of the model. Furthermore, the difference in $VO_{2peak}$ was smaller than the standard error of measurement (SEM 138 $mL \cdot min^{-1}$ (8.5%)) in a test-retest reliability study of $VO_{2peak}$ in adult patients with CF in a severe disease status (mean $FEV_1$ 52% of predicted, age 26.9±6.0).[23] Linear regression analysis in the validation group with $VO_{2peak}$ as the dependent determinant revealed the same parameters as independent determinants with a comparable $R^2$ of .85 versus .91 in the reference group.

The results of this study have implications for clinical practice in adolescents with CF. When gas analysis is not available, $W_{peak}$ from the Godfrey protocol and sex may serve as clinical valid predictors of $VO_{2peak}$ in adolescents with CF in various disease states. The implementation of the Godfrey protocol and this equation in clinical practice might help to increase the utilization of exercise testing and measuring physical fitness in this patient group.

We found high positive predictive values and high negative predictive values for the model to assign individual patients to different prognosis clusters. Only the positive predictive value for a low aerobic capacity ($VO_{2peak}$ <32 $ml \cdot min \cdot kg^{-1}$) was low (50%), which can be explained by the low prevalence of a low aerobic capacity in the validation group (n=1).[24] As Pianosi et al found a $VO_{2peak}$ < 32 $ml \cdot min \cdot kg^{-1}$ to be associated with a 10 year mortality of 50% and a $VO_{2peak}$ > 45 $ml \cdot min \cdot kg^{-1}$ to be associated with a 10 year mortality of 0%, the mean difference in $VO_{2peak}$ of our model (107 $ml \cdot min^{-1}$) is also quite accurate in a prognostic point of view.[24] In our validation group with a mean weight of 50.44 kg, the difference in $VO_{2peak}$ between "good" and "bad" prognostic groups would be 655.7 $ml \cdot min^{-1}$ (50 kg * (45$ml \cdot min \cdot kg^{-1}$ – 32 $ml \cdot min \cdot kg^{-1}$)), whereas the SEE of the model is 107 $ml \cdot min^{-1}$. Furthermore, the model is designed in a group of patients of varying prognosis (95% prediction interval between 1770 (~ 23 $ml \cdot min \cdot kg^{-1}$) and 2548 $mL \cdot min^{-1}$ (~50$ml \cdot min \cdot kg^{-1}$). Additionally, calculated with the mean weight of the validation group, the predicted group means $VO_{2peak}$ and estimated $VO_{2peak}$ were both within the same prognosis cluster of Pianosi et al (predicted $VO_{2peak}$ 44.23 $ml \cdot min \cdot kg^{-1}$ versus measured $VO_{2peak}$ 42.13 $ml \cdot min \cdot kg^{-1}$). However, we would like to emphasise that Pianosi build his model on a patient population measured between 1991 and 1996, whereas we used data from a 1996-2006 cohort. Within this different time-frame the quality of CF care has increased considerably due to progression in consensus and evidence-based medicine.[25, 26] This could have consequences for prognostic values of criteria, for example, the development of the $FEV_1$<30%$_{pred}$ criterion, which indicated a median two year life expectancy based on a 1977-1989 cohort [27], while its expectancy increased to a median five year survival in a 1990-2003 cohort.[25] This highlights the caution which should be taken in using the cut-off values reported in older literature such as of e.g. Pianosi et al.

With the prognostic value of exercise testing and especially $VO_{2peak}$, annual follow-up of exercise capacity is important to identify individuals who are at risk for poorer prognosis and identify those who may benefit from more intense therapy. [28] However, a future study should also focus on the further validation of the developed model to predict $VO_{2peak}$ in patients with more advanced CF when more exercise limiting mechanisms are involved. Furthermore, as some (variable) level of impairment in $VO_{2peak}$ is to be expected in patients with chronic conditions, it may be clinically helpful to interpret the achieved level of exercise capacity in comparison with what would be usual/expected given the patient's age, gender, and underlying diagnosis.[29] Therefore, future studies should also focus on obtaining CF specific reference values for cycle ergometer exercise testing as has been done for patients with other chronic conditions.[29, 30]

When using the reference equation to estimate $VO_{2peak}$, and in the absence of measured RER, we suggest to use a peak heart rate criterion of > 180 bpm in adolescents beside the subjective signs, to asses whether an individual performed a maximal effort during cycling.[21] Hence, care should be taken to consider a test as submaximal when the peak heart rate is below 180 bpm as a ventilatory limitation can limit the heart rate to increase to maximal levels, as supported by previous work in our laboratory where we found significant lower peak heart rates in adolescents with CF with evident static hyperinflation. [31]

In conclusion, we have shown that peak work rate obtained using the Godfrey protocol and gender can be clinically used as a simple and valid alternative for the estimation of $VO_{2peak}$ in adolescents with CF in mild to moderate disease states in situations where it is not possible to formally measure $VO_{2peak}$ with gas analysis.

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PART 2 EXERCISE LIMITING MECHANISMS

# Chapter 5

# Is static hyperinflation a limiting factor during exercise in adolescents with cystic fibrosis?

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## ABSTRACT

Background: Increased work of breathing is considered to be a limiting factor in patients with Cystic Fibrosis (CF) during exercise. We hypothesized that static hyperinflated adolescents with CF are more prone to a ventilatory limited exercise capacity than non-static hyperinflated adolescents with CF.

*Methods*: Exercise data of 119 adolescents with CF [range 12-18 yrs.], stratified for static hyperinflation, defined as ratio of residual volume to total lung capacity (RV/TLC) > 30%, were obtained during bicycle ergometry with gas analysis and analyzed for ventilatory limitation.

*Results*: Static hyperinflation showed a significant, though weak association (Φ 0.38; $p < .001$) with a ventilatory limited exercise capacity (Breathing Reserve Index at maximal effort > 0.70; $FEV_1$ < 80% predicted and $VO_{2peak}$ < 85% predicted). Analysis of association for increasing degrees of hyperinflation showed an increase to Φ .49 ($p < .001$) for RV/TLC > 50%. In adolescents with static hyperinflation, peak work rate ($W_{peak}$; 3.1±0.7 Watt/kg (75.1±17.3 % of predicted), peak oxygen uptake ($VO_{2peak}$/kg; 39.2±9.2 ml/min/kg (91.0±20.3 % of predicted), and peak heart rate ($HR_{peak}$; 176±19 bpm) were significantly ($p < .05$) decreased when compared with non-static hyperinflated adolescents ($W_{peak}$ 3.5 ± 0.5 Watt/kg (81.4±10.0 % of predicted)); $VO_{2peak}$/kg; 43.1±7.5 ml/min/kg (98.0±15.1 % of predicted) and $HR_{peak}$ 185±14 bpm). Additionally, no difference was found in the degree of association of $FEV_1$ (%) and RV/TLC (%) with $VO_{2peak}/kg_{pred}$ and $W_{peak}/kg_{Pred}$, but we found the RV/TLC (%) to be a slightly stronger predictor of $VO_{2peak}/kg_{pred}$ and $W_{peak}/kg_{Pred}$ than $FEV_1$ (%).

*Conclusion*: These results indicate that the presence of static hyperinflation in adolescents with CF by itself does not strongly influence ventilatory constraints during exercise and that static hyperinflation is only a slightly stronger predictor of $W_{peak}/kg_{Pred}$ and $VO_{2peak}/kg_{Pred}$ than airflow obstruction ($FEV_1$ (%)).

## INTRODUCTION

Limitation of exercise capacity in adolescents with cystic fibrosis (CF) has a multi-factorial cause. Reduced lung function and muscle mass are known to be most important factors leading to a limited exercise capacity.[1, 2] A decreased muscle mass reduces skeletal muscle function, including respiratory muscle strength, in adults with CF.[3] Moreover, in children with CF a decreased skeletal muscle strength [4, 5] and endurance [6] have been reported, even when corrected for a decreased lean body mass or lung function.[4-6] This points to a possible intrinsic abnormality in muscle oxygen uptake in patients with CF, however, currently there is no firm evidence available.[7-11]

Due to continuous airflow obstruction, as reflected by a decreased forced expiratory volume in one second ($FEV_1$), and dynamic hyperinflation, as reflected by a decreasing inspiratory capacity (IC) during exercise,[12] children with CF develop a rapid breathing pattern during exercise with a concomitant increase in the work of breathing (WOB) [13-15] and oxygen cost.[13] A decreased inspiratory muscle function (strength and endurance) that has been observed in patients with CF will lead to a faster inspiratory muscle fatigue during exercise, which contributes to the reduced exercise capacity.[16, 17]

It seems that there is an interrelationship between lung function, muscle mass, energy expenditure, (respiratory) muscle function and exercise capacity in patients with CF.[18]

The objective of the current study was to investigate whether static hyperinflation makes adolescents with CF more prone to a ventilatory limitation during exercise. We hypothesized that adolescents with static hyperinflation are more prone to a ventilatorily limited exercise capacity than non-static hyperinflated adolescents with CF. Furthermore, we questioned if the amount of static hyperinflation (RV/TLC (%)) is a stronger predictor of exercise capacity than the degree of airflow obstruction $FEV_1$ (%pred).

# METHODS

## Subjects

Adolescents with CF (n = 119) of the Cystic Fibrosis Center of the University Children's Hospital and Medical Center Utrecht, the Netherlands, were measured for body weight, height, lung function and exercise capacity as part of routine assessment measures at the annual medical check-up. All measurements were part of usual care, according to the policy of the medical ethical committee of the University Medical Center Utrecht, ethical approval and informed consent were not obliged. Data of the initial test of each participant (between 1998 and 2006) were selected for this study. Participants were stratified into static hyperinflated and non-static hyperinflated. Conform previous literature about children with asthma, we defined a ratio of residual volume to total lung capacity (RV/TLC), after using a bronchodilator, higher than 30% as moderate to severe hyperinflation. [19] Analysis for increasing degrees of static hyperinflation was performed by including only patients with a certain level of static hyperinflation in the analysis (RV/TLC > 30%, > 35%, > 40%, >45% and >50%).
The definition used for determination of a ventilatory limitation during Cardio Pulmonary Exercise Test (CPET) was previously used by Sexauer et al, including: [1] Breathing Reserve Index at maximal effort > 0.70 (Calculated as peak minute ventilation ($VE_{peak}$) divided by maximal voluntary ventilation (MVV) were MVV is calculated as 35 x $FEV_1$), [2] a $FEV_1$ < 80% predicted and [3] a reduced exercise capacity, defined as $VO_{2peak}$ < 85% predicted.[20]

## Spirometry

Spirometry and body plethysmography were performed before and after bronchodilation with salbutamol (800 ug), using a pneumotach system and a volume-constant plethysmograph (Master Lab system, E. Jaeger, Würzburg, Germany).
Lung function measurements included total lung capacity (TLC), residual volume (RV) and forced expiratory volume in 1 second ($FEV_1$). The results were compared with predicted values for healthy subjects matched for age, body height, and gender.[21]

## Cardiopulmonary exercise test (CPET)

Exercise capacity was assessed using a progressive cardiopulmonary exercise test (CPET). CPET, after brochodilation with salbutamol, performed on an electronically braked cycle ergometer (Jaeger physis; Carefusion, Houten, The Netherlands). The seat height was adjusted to the participant's comfort and leg length. Participants rested until all measured

variables were stable. Cycling started at a workload of 0 W; the workload was incremented with 15 W/min until the patient stopped due to volitional exhaustion. The workload which could be overcome for the last 30 seconds prior to exhaustion was considered to be the $W_{peak}$. Determination if a participants' effort was maximal was based on subjective and objective criteria. Subjective criteria are described as "unsteady biking", "sweating", "facial flushing" and "clear unwillingness to continue despite encouragement". Objective criteria were: [1] peak heart rate (HR) > 95% $HR_{predicted}$ (210-age) and [2] respiratory exchange ratio (RER) > 1.00. Based on previous literature, we defined that a participant had to meet the subjective criteria and at least 1 out of two objective criteria for the test to be considered of maximal effort and character.[22] Participants breathed through a mask that was connected to a calibrated metabolic cart (Oxycon pro, Carefusion, Houten, The Netherlands). Expired gas was passed through a flow meter, oxygen analyzer, and a carbon dioxide analyzer. The flow meter and gas analyzer were connected to a computer, which calculated breath-by-breath minute ventilation (VE), oxygen uptake ($VO_2$), carbon dioxide production ($VCO_2$) and respiratory exchange ratio (RER) from conventional equations. Relative peak oxygen uptake ($VO_{2peak}$/kg) was calculated by dividing $VO_{2peak}$ by total body mass. Heart rate was monitored continuously by a 3-lead electrocardiogram (Hewlett-Packard, Amstelveen, Netherlands).



## Reference values

Reference values for $VO_{2peak}$ and $W_{peak}$ from healthy children and adolescents were obtained from previously studied Dutch children and adolescents.[23, 24]

## Statistical analysis

Data were expressed as mean ± SD. Data were analyzed using SPSS 15.0 for Windows and tested for normality with the Kolmogorov-Smirnov Test. An alpha value of 0.05 was considered as statistically significant. Possible differences between groups in the CF-population were analyzed using one-way ANOVA when normally distributed and with the Mann-Whitney U-Test when not normally distributed. Dichotomy variables were tested for association using the phi coefficient (Φ), and tested for significance using Chi-square test. Prognostic value of $FEV_1$ and RV/TLC for exercise capacity was analyzed using standard multiple regression analysis.

## RESULTS

*Study Group Demographics*

After determination of maximal effort and screening for completeness of data set, in total 119 adolescents, 50 females and 69 males were included.

Mean age was 13.8 years ± 1.7 (range 12-18 years), with a mean $FEV_1$ of 82.9% ± 20.9 (% predicted). $FEV_1$ and anthropometric values did not differ according to gender (Table 1). All measurements were obtained after bronchodilator.

**Table 1.** Demographic characteristics

| | Female (n=50) | Male (n=69) | Total (n=119) |
|---|---|---|---|
| Age (yrs) | 13.7±1.5 | 13.8±1.8 | 13.8±1.7 |
| Heigth (cm) | 156.6±7.7 | 159.1±11.4 | 158.1±10.1 |
| BM (kg) | 43.8±8.1 | 44.3±10.7 | 44.1±9.6 |
| BMI ($kg/m^2$) | 17.8±2.1 | 17.2±2.0 | 17.4±2.1 |
| RV/TLC (%) (before Ventolin) | 36.3±9.8 | 34.4±12.8 | 35.2±11.6 |
| RV/TLC (%) (after Ventolin) | 33.2±9.6 | 32.1±11.7 | 32.5±10.9 |
| $FEV_1$ (%Pred.) (before Ventolin) | 80.2±19.2 | 76.8±22.1 | 78.2±20.9 |
| $FEV_1$ (%Pred.) (after Ventolin) | 84.8±20.1 | 81.5±21.5 | 82.9±20.9 |
| $HR_{rest}$ (beats/min) | 102.1±20.6 | 100.2±14.5 | 101.0±17.3 |
| $RER_{rest}$ ($VCO_2/VO_2$) | 0.92±0.09 | 0.91±0.08 | 0.91±0.08 |
| $VO_{2peak}$ (L/min) | 1.6±0.4[c] | 1.9±0.5 | 1.8±0.5 |
| $VO_{2\ peak}$ (ml/min/kg) (%Pred.) | 36.5±6.3[a] (94.5±16.7%) | 44.2±8.8 (94.0±19.6%) | 41.0±8.7 (94.2±18.4%) |
| $W_{peak}$ (Watt) | 133.3±27.4[c] | 152.7±48.0 | 144.6±41.6 |
| $W_{peak}$ (Watt/ kg) (%Pred.) | 3.1±0.5[a] (79.4±12.3%) | 3.5±0.7 (76.9±16.3%) | 3.3±0.7 (77.9±14.8%) |
| $HR_{peak}$ (beats/min) | 180.1±15.1 | 179.7±19.0 | 179.9±17.4 |
| $RER_{peak}$ ($VCO_2/VO_2$) | 1.18±0.09[b] | 1.13±0.08 | 1.15±0.09 |
| $VE_{peak}$ (L/min) | 62.2±15.6[d] | 70.4±22.7 | 67.0±20.3 |

[a] Significant difference between gender groups ($p < 0.001$); [b] Significant difference between gender groups ($p < 0.01$); [c] Significant difference between gender groups ($p < 0.05$): Non parametric tested with Mann-Whitney U-test; [d] Significant difference between gender groups ($p < 0.05$); Values are presented as means ± SD. Abbreviations: BM=body mass; BMI=body mass index; RV/TLC=ratio residual volume/total lung capacity; $FEV_1$=forced expiration volume in one second; HR = heart rate; RER = respiratory exchange ratio ($VCO_2/VO_2$); $VO_{2\ peak}$ = peak oxygen uptake; $W_{peak}$ = peak work rate; $VE_{peak}$=minute ventilation at maximal effort

## Static hyperinflation versus non-static hyperinflation

Overall, 54 (40.3%) patients were non-static hyperinflated (20♀; 34♂; RV/TLC (%) 23.4±3.4; $FEV_1$ (%pred) 97.0±13.6), whereas 65 (48.5%) patients were identified as static hyperinflated (30♀; 35♂; RV/TLC (%) 40.1±9.0; $FEV_1$ (%pred) 71.4±18.7). Peak exercise parameters and ventilatory parameters in static hyperinflated and non-static hyperinflated adolescents with CF are shown in Table 2. $VO_{2peak}$/kg, $W_{peak}$/kg, $VE_{peak}$ and $HR_{peak}$ were all significantly lower in static hyperinflated patients compared to the non-hyperinflated patients ($p < 0.05$). Corrected for age and gender, the differences in $VO_{2peak}/kg_{Pred}$ (98.0±15.1 % in non-static hyperinflated and 91.0±20.3 % in static hyperinflated patients; $p < .05$), and in $W_{peak}/kg_{Pred}$ (75.1±17.3 (%) in static hyperinflated and 81.4±10.0 (%) in non-static hyperinflated patients; $p < .05$) remained (Table 2).

**Table 2.** Exercise capacity in static hyperinflated and non-static hyperinflated patients with CF

| | Static hyperinflation (N = 65) | Non-static hyperinflation (N = 54) |
|---|---|---|
| $HR_{peak}$ (beats/min) | 176±19 | 185±14[a] |
| $RER_{peak}$ ($VCO_2/VO_2$) | 1.14±0.09 | 1.17±0.08 |
| $VE_{peak}$ (L/min) | 59.5±17.2 | 76.0±20.2[b] |
| $VO_{2peak}$/kg (ml/min/kg) | 39.2±9.2 | 43.1±7.5[b] |
| $VO_{2peak}/kg_{Pred}$ (%) | 91.0±20.3 | 98.0±15.1[b] |
| $W_{peak}$/kg (Watt/kg) | 3.1±0.7 | 3.5±0.5[b] |
| $W_{peak}/kg_{Pred}$ (%) | 75.1±17.3 | 81.4±10.0[b] |

Values are presented as means ± SD. Abbreviations: $HR_{peak}$ = peak heart rate; $RER_{peak}$ = respiratory exchange ratio at peak exercise; $VE_{peak}$ = peak minute ventilation; $VO_{2\ peak}$/kg = peak oxygen uptake per kilogram body mass; $W_{peak}$/kg = peak work rate per kilogram body mass; [a] $p < 0.05$ Non parametric tested with Mann-Whitney U-test, [b] $p < 0.05$.

## Ventilatory versus Non-ventilatory limitation

Twenty (29.4%; 7♀; 13♂) out of the 65 patients with static hyperinflation were ventilatorily limited during exercise while only 1 (1♂) of the 54 patients without static hyperinflation was ventilatorily limited during exercise. Phi coefficient (Φ) between ventilatory limitation and static hyperinflation was 0.38 ($p < 0.001$; see Table 3). Furthermore, for increasing degrees of hyperinflation, Phi coefficient increased to .52 ($p < .001$) in the RV/TLC range 30% - 50%. (Table 3)

**Table 3.** Correlation between static hyperinflation and ventilatory limitation for different degrees of hyperinflation

| | Phi coefficient | p-value |
|---|---|---|
| RV/TLC > 30% | .38 | <.001 |
| RV/TLC > 35% | .52 | <.001 |
| RV/TLC > 40% | .50 | <.001 |
| RV/TLC > 45% | .50 | <.001 |
| RV/TLC > 50% | .49 | <.001 |

Values are Phi correlation coefficients with p-value. Abbreviations: RV/TLC = residual volume / total lung capacity.

## Lung function and exercise capacity

As presented in Figure 1, baseline $FEV_1$ (%pred) after bronchodilator, showed a fair degree of association with $VO_{2peak}/kg_{Pred}$ and $W_{peak}/kg_{Pred}$ ($r = .44$ and $r = .46$ respectively (both $p < .001$), where RV/TLC, after bronchodilator, showed more variable degrees of association ($r = -.43$ and $r = -.47$ respectively (both $p < .001$) after bronchodilator). There was a strong association between $FEV_1$ (%pred) and RV/TLC after bronchodilator ($r = -.84$; $p < .001$).
Multiple linear regression showed that, compared to FEV1 (%pred), RV/TLC (%) was a somewhat stronger predictor for $W_{peak}/kg_{Pred}$ (FEV1 (%pred) B .161 and ß .227 (p=.135)); RV/TLC (%) B -.371 and ß -.273 ($p = .073$)) and $VO_{2peak}/kg_{Pred}$ (RV/TLC (%) B -.343 and ß -.203 ($p = .188$); $FEV_1$ (%pred) B .239 and ß .272 ($p = .078$)).

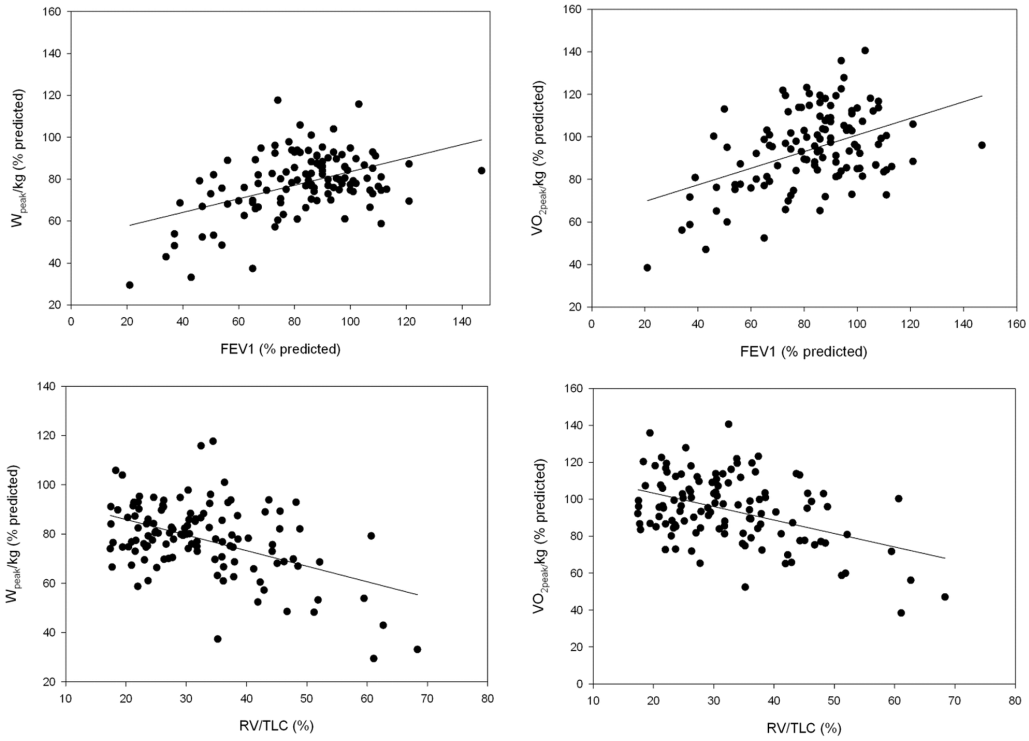


**Fig. 1.** Associations between lung function parameters and exercise capacity.

## DISCUSSION

We hypothesized that adolescents with CF with static hyperinflation are more prone for a ventilatorily limited aerobic exercise capacity compared with non-static hyperinflated adolescents. We found a significant, but weak association (Φ 0.38; $p < 0.001$) between static hyperinflation (RV/TLC > 30%) and ventilatory limitation at peak exercise. This indicates that the presence of static hyperinflation in adolescents with CF by itself does not strongly influence ventilatory constraints during exercise, which is in line with previous research. [20] Sexauer et al found an odds ratio of 0.96 ($p = 0.76$) for the RV/TLC ratio at rest as a weak non-significant predictor for ventilatory limitation in adults with CF.[20] Confirmative results were found in a study among adult COPD patients, were the change in inspiratory capacity (IC) during exercise, reflecting dynamic hyperinflation, has been shown to be superior to static hyperinflation (resting IC) in estimating exercise tolerance.[25]
Moreover, after analysis of the association between the degrees of static hyperinflation and a ventilatorily limited exercise capacity, the correlation coefficient slightly increased from .38 to .49 for RV/TLC > 30% and > 50%, respectively.
Additionally, we found no difference in the degree of association of baseline $FEV_1$ (%pred) and RV/TLC (%), after bronchodilator, with $VO_{2peak}/kg_{Pred.}$ and $W_{peak}/kg_{Pred}$, however, RV/TLC (%) was a slightly stronger predictor of $VO_{2peak}/kg_{Pred.}$ and $W_{peak}/kg_{Pred}$ than $FEV_1$ (%pred).
A point of discussion in the present study is the cut-off point in breathing reserve used to determine ventilatory limitation. Prioux et al suggested a ventilatory reserve at peak exercise of 20% (MVV-VE / MVV x 100%), with a corresponding breathing reserve 0.8, in 11- year- old children, which increased to 30% (corresponding breathing reserve 0.7) at the age of 16 years. The mean age of our patients is 14 years, which could have influenced the prevalence of ventilatory limitation as we have used breathing reserve > 0.7 as cut-off point.[26] Furthermore, due to the narrow age range of our population, the present results could not be extrapolated to patients that are younger or older.
Based on the results we conclude that the presence of static hyperinflation after bronchodilator (RV/TLC > 30%) in adolescents with CF by itself does not strongly influence ventilatory constraints during exercise and that static hyperinflation, as reflected by RV/TLC (%), is only a slightly stronger predictor of $W_{peak}/kg_{Pred}$ and $VO_{2peak}/kg_{Pred}$ than the $FEV_1$ (%pred), which is only reflecting the degree of airflow obstruction. The decreased exercise capacity in static hyperinflated adolescents could be explained by faster termination of peak exercise due to preliminary inspiratory muscle fatigue. The preliminary inspiratory muscle fatigue could be induced by the development of dynamic hyperinflation,[14, 15, 24] which increases work [12-15] and oxygen cost of breathing [13] and causing intrapulmonary gas trapping and ventilation/perfusion mismatching,[27] which make a patient with CF more susceptible to ventilatory limitation during exercise.[15] Moreover,

the greater fatigability of the inspiratory muscles could hypothetically induce a reflex vasoconstriction in the peripheral locomotor muscles and thereby compromises blood flow to the exercising limbs.[17] Furthermore, the increase in work of breathing in patients with CF as a possible factor in ventilatorily limited exercise capacity could theoretically be elicited by the development of dynamic hyperinflation during exercise, instead of the presence of static hyperinflation at rest.

Beside the $FEV_1$ and possible dynamic hyperinflation, other factors, such as nutritional status, muscle mass, respiratory and peripheral muscle strength and habitual daily physical activity are also important predictors of exercise capacity.[1, 2, 4, 28]

After all we suggest that, in future research, beside standard anthropometric and lung function measures, dynamic hyperinflation and flow-volume curves during exercise should be measured to facilitate a better understanding of the role of increased work of breathing as possible limiting factor in the exercise capacity of patients with CF.

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# Chapter 6

# Exercise oxidative skeletal muscle metabolism in adolescents with cystic fibrosis

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## ABSTRACT

*Background*: Some evidence exists for intrinsically impaired skeletal muscle function in CF which may be caused by poor oxygenation. We tested the hypothesis that abnormalities in oxygenation and/or muscle oxidative metabolism contribute to exercise intolerance in adolescents with CF.

*Methods*: Ten clinical stable adolescents with CF and ten healthy age-matched controls (HC) performed two incremental exercise tests. Two complementary non-invasive techniques (Near-Infrared Spectroscopy (NIRS) and $^{31}$Phosphorus magnetic resonance spectroscopy ($^{31}$P MRS)) were used separately.

*Results*: All patients with CF were non anaemic without signs of low grade or chronic systemic inflammation. No statistically significant ($p>0.1$) differences in peak workload and peak oxygen uptake per kilogram lean body mass were found between CF and HC. No differences were found between CF and HC in bulk changes of quadriceps phosphocreatine (PCr) ($p = .550$) and inorganic phosphate (Pi) ($p = .896$) content and pH ($p = .512$) during exercise. We found statistically identical PCR resynthesis kinetics during recovery for CF and HC ($p = .53$). No statistically significant difference in peak exercise arbitrary unit for total haemoglobin content was found between CF and HC ($p = .66$).

*Conclusion*: Our findings suggest a normal oxidative exercise metabolism and normal oxygenation kinetics in clinical stable adolescents with CF.

## INTRODUCTION

Several mechanisms, as pulmonary, cardiac and peripheral skeletal muscle function, contribute to the reported limited exercise capacity in patients with Cystic Fibrosis (CF) [1]. Impaired skeletal muscle function may be caused by poor oxygenation of the skeletal muscles, possibly due to impaired blood flow during exercise with excessive ventilatory demands [2]. Other studies however reported that Cystic Fibrosis Transmembrane conductance Regulator (CFTR)-deficient skeletal muscles demonstrate functional abnormalities primarily during periods of (increased) inflammation, leading to increased muscle weakness [3]. This is in agreement with the impaired exercise capacity in patients with CF colonized with Pseudomonas aeruginosa [4].

A number of studies reported evidence for intrinsically impaired skeletal muscle function, independent of lung function and muscle mass [5-8]. Recently, the CFTR chloride channel gene is expressed in human skeletal muscle cells [3, 9]. However, there is no consensus about either its exact localization in the muscle cell or any potential impact of a mutated CFTR channel on muscular contractile performance during exercise [5-8, 10, 11]. There is some evidence for altered proton handling and reduced mitochondrial function in CF muscle [5]. A recent study reported attenuated mitochondrial function in non-skeletal muscle cells [12]. In muscle, 31P magnetic resonance spectroscopy (31P MRS) studies of oxidative metabolism during exercise in CF patients revealed slight abnormalities in oxidative work performance [8] and phosphocreatine (PCr) recovery [5, 8].

The present study was performed to test the hypothesis that abnormalities in oxygenation and/or muscle oxidative metabolism contribute to exercise intolerance in CF. We studied the oxidative metabolism in upper leg muscles in children and adolescents with moderate CF performing two incremental bicycle exercise tests. Two complementary non-invasive techniques (Near-Infrared Spectroscopy (NIRS) and $^{31}$Phosphorus magnetic resonance spectroscopy ($^{31}$P MRS)) were used during two separate test sessions.



## METHODS

### Study design

Ten patients with CF and ten healthy age-matched controls (HC) were studied. Patients with CF were between twelve and eighteen years of age and all in Class I-III CFTR mutation. The patients with CF were free from acute exacerbation (no extra medication or in-hospital treatment for pulmonary of gastro-intestinal exacerbations < last three weeks) with the forced expiratory volume in one second ($FEV_1$ ($L{\cdot}min^{-1}$)) above 80% of predicted [13], and the oxygen saturation in rest ($SpO_2$ (%)) above 94%.

For both the CF and the HC (age-matched) participants, needed to be free from constraints in performing a maximal exercise test in a magnetic resonance (MR) scanner. Possible contra-indications for in magnet cycling were identified prior to testing by standardized questionnaires. The research protocol was approved by the medical ethics committee of the University Medical Center Utrecht. All participants, CF and HC, and (when < 18 years of age) their parents gave written informed consent.
The participants visited the hospital twice separated by at least two days. In the first session the thigh muscles of patients with CF and HC were measured with Near-Infrared Spectroscopy (NIRS) during rest, incremental cycling exercise, and recovery. In the second session they performed the same incremental cycling exercise protocol during a $^{31}$Phosphorus magnetic resonance spectroscopy ($^{31}$P MRS). The tests were done in the University Medical Center Utrecht, at the Child Development and Exercise Center and the Department of Radiology.

## Spirometry, anthropometrics and laboratory measurements

Lung function (MicroLoop, PT-Medical, Leek, the Netherlands) and anthropometric values, using an electronic scale (Seca, Birmingham, United Kingdom) and a stadiometer (Ulmer stadiometer, Prof. E. Heinze, Ulm, Germany), were measured before both test sessions. Percentage body fat and subsequent lean body mass (LBM) were determined by measuring subcutaneous fat of the biceps, triceps, subscapular, and supra-iliac regions with a Harpenden skinfold caliper. Body density and percentage body fat were then calculated as recently described by Bongers et al [14]. Participants completed the Habitual Activity Estimation Scale questionnaire two weeks before the first test session [15].
Laboratory data of venous blood sampling, measured and collected during regular patient controls, of C-reactive protein (CRP), total IgG and haemoglobin (Hb) levels were retrieved from the hospital electronic database. We used the samples which have been obtained the most recent before the exercise tests data. Acute low-grade systemic inflammation was defined as C-reactive protein (CRP) levels above 0.5 mg·dL (16), whereas high IgG-total levels were used as a representative of chronic inflammation (4). Anaemia was defined as hemoglobine (Hb) levels below 8.6 mmol/l for male and below 7.4 mmol/l for female participants. We used the cut-off values and reference values from the Clinical Chemistry Laboratory of the University Medical Center Utrecht. (*Reference values, Department of Clinical Chemistry and Haematology, UMC Utrecht, Version 010, 2012*)

## Exercise Protocol

Both exercise tests were performed on a specialized MR-compatible bicycle ergometer (17). Subjects were asked to lay supine on the bicycle ergometer, and their feet were fixated onto the pedals of the ergometer. The upper body position was under maximal upright angle (typically 40°), using a wedge-shaped support cushion. Before the cycling started, subjects rested in supine position for 5 minutes to ensure a stable resting muscle metabolism. The actual measurement started with one minute of unloaded cycling to familiarize the subject with the exercise, including stabilizing his upper body position by pulling on the handlebar ropes and testing proper function of all components. The desired cycling rate (80 revolutions per minute) was communicated to the subject by audio feedback of a metronome signal. Subsequently, subjects performed an incremental exercise protocol (estimated duration 8-10 minutes), which step increments protocol was tailored to provide exhaustion between 8-10 minutes. The workload increments were 0.3 kg/min for males and 0.2 kg/min for females. Recovery measurements were obtained for 5 minutes after exercise had ceased.

## $^{31}$P-MRS protocol

Subjects were positioned supine and feet-first in the MR scanner (1.5 T Intera, Philips Healthcare, Best, the Netherlands) and attached to the MR-compatible cycle ergometer. A 6-cm 31P surface coil (P-60; Philips Healthcare, Best) was fixated over the medial head of the quadriceps muscle by Velcro strips. Subjects then performed a short bout of unloaded cycling to familiarize them with the exercise regimen. First, a set of scout 1H images of the upper leg was obtained using the body 1H coil for localized shimming of the magnet over the medial head of the quadriceps for 31P spectroscopic acquisitions. Next, resting 31P MR spectra were acquired with a 90° adiabatic excitation (AHP) pulse (16 free induction decays; FIDs) and repetition times (TR) of 20,000 and 3,000 ms, respectively, to establish saturation correction factors for the TR of 3,000 ms that was used in all ensuing acquisitions. During incremental exercise, 31P MR spectroscopic data acquisition was synchronized with the cyclic motion of the upper leg as described by Jeneson in 2010. Two FIDs were averaged per spectrum yielding a time resolution of 6 s in the dynamic datasets acquired during exercise and subsequent recovery.

## $^{31}$P MRS Data processing and analysis

PCr, Pi, and ATP resonances were fitted in the time domain using the AMARES algorithm in the jMRUI software package. Absolute concentrations were calculated after correction for partial saturation and assuming adenine nucleotide and creatine pool sizes of 8 and 42

mM, respectively [18]. Intracellular pH was calculated from the chemical shift difference between the Pi and PCr resonances (18). Free ADP concentrations ([ADP]) and the molar Gibbs free energy of cytosolic ATP hydrolysis ($\Delta G_p$) at rest and at maximal exercise were calculated as described by Jeneson in 1997. The mitochondrial response function to ADP concentration changes and the thermodynamic flow-force function of ATP free energy transduction were determined by nonlinear curve-fitting (Microcal Origin 6.0, OriginLab, Northampton MA, USA) of a sigmoidal function to the covariation of [ADP] and $\Delta G_p$ with exercise workload, respectively, as described elsewhere [19]. In the analysis, a proportional relation between workload and mitochondrial ATP synthesis rate was assumed [20]. The kinetics of PCr recovery following exercise were determined by nonlinear curve-fitting of a mono-exponential function to the PCr time course [21].

## NIRS protocol

During NIRS measurements, the participants lay supine on a MR-compatible cycle ergometer. The measurements were performed according to a previously used protocol in our exercise laboratory [22]. The probes of a single distance continuous wave photometer with two channels (OXYMON; Artinis, Zetten, the Netherlands) were fixed on the VM of the dominant leg of the participant. The VL probe was located on one third of the distance from the lateral epicondyle to the greater trochanter of the femur. The VM probe was located in the same transversal plane, but at the medial part of the leg. The light source and detector was housed in a holder, with a constant distance of 3.0 centimetres between, which an average measurement depth of approximately 1.5 centimetres. Near-infrared light was emitted at two wavelengths (775 nm and 850 nm).
Since the length of the light beam that travels through the tissue is longer than the distance between the source and the detector due to the scattering effects of different tissue layers (skin, adipose tissue thickness, and muscle), a differential path-length factor (DPF) set at 4.0 had to be included to calculate the path-length. To prevent the probes shifting on the skin, the probes were fixed by tape on the skin. To reduce the intrusion of stray light and loss of transmitted light from the field of examination, a black cloth was placed at the location of the NIRS device on the leg. Thereafter, an elastic bandage was wrapped around the leg a few times to further minimize movement of the probes. Changes in deoxyhemoglobin, oxyhemoglobin and total haemoglobin concentrations ([HHb], [$O_2$Hb] and [tHb]) from baseline were expressed in μM. Data were sampled and displayed in real time at a frequency of 50 Hz from the start of the rest to the end of the recovery period. The data were analysed after filtering by a Gaussian filter and NIRS outcome measures were normalized to arbitrary units (AU), as described by Habers et al, to allow comparisons between groups [22]. This normalization procedure involved averaging the values of Δ[O2Hb], Δ[HHb] and Δ[tHb] over the last 30 s of the resting

period before exercise and assigning them a value of 0 arbitrary units (AU). Next, Δ[O2Hb], Δ[HHb] and Δ[tHb] during the recovery phase were averaged over a 10-s period, and the maximal values were assigned a value of 1 AU. All values between these two time-points were normalized to this scale. Normalized Δ[O2Hb], Δ[HHb] and Δ[tHb] values were thus dependent on their representative maximum values reached during peak exercise (Δ[HHb]) and/or recovery (Δ[tHb] and Δ[O2Hb]).

### Statistical analysis

Data were analyzed with SPSS 15.0.for Windows. In all quantitative variables the Kolmogorov-Smirnov-test for normality was performed. An alpha level of $p< 0.05$ was set as statistical significant. Two-tailed unpaired Student's t-tests were used to compare both groups (HC and CF) [23]. For the end-exercise PCr and pHi data, obtained during MR cycling, we only used the datasets of participants who cycled "maximal" (> 75% of $W_{peak}$) attained in the first test with NIRS. Statistical analysis was performed by the single-blinded researcher (MW).

## RESULTS

All 20 subjects completed both exercise tests without any adverse events. We found no significant differences in anthropometric and baseline values between patients with CF and HC. (Table 1) Patients with CF and HC were comparably physically active during daily life, except for the HAES domain "very active" (CF 212±109 minutes versus HC 335±149 minutes; $p = .049$).

**Table 1.** Baseline characteristics

| Variable | CF (mean ± SD) | HC (mean ± SD) | p-value |
|---|---|---|---|
| Age (years) | 13.8±1.3 | 13.7±1.1 | .944 |
| Height (cm) | 163.0±9.3 | 165.6±10.1 | .584 |
| Weight (kg) | 49.0±6.9 | 56.2±10.8 | .118 |
| BMI (kg/m$^2$) | 18.3±0.9 | 20.3±2.7 | .054 |
| Fat percentage (%) | 17.5±2.7 | 20.8±6.2 | .156 |
| Lean body mass (kg) | 41.2±5.3 | 44.7±8.4 | .292 |
| $FEV_1$ (%pred.) | 92.8±14.6 | 90.3±13.4 | .717 |
| Gender | 5♀ 5♂ | 5♀ 5♂ | 1.0* |
| Hb (mmol·L) | 8.4±0.7 [7.1-9.4] | n.a. | n.a. |
| CF mutation | ΔF508 homozygote n= 3<br>ΔF508 heterozygote n= 7<br>(Class I n= 5; Class II n=3;<br>Class III n=2; Class IV and V n=0) | n.a. | n.a. |
| Habitual daily activity level (min/day) | Inactive 40±42<br>Somewhat inactive 719±292<br>Somewhat active 439±263<br>Very active 212±109 | Inactive 100±167<br>Somewhat inactive 560±293<br>Somewhat active 544±391<br>Very active 335±149 | .276<br>.241<br>.488<br>.049 |

BMI = body mass index (kg/m$^2$); $FEV_1$ (l/min) = forced expiratory volume in one second; Hb = haemoglobin
* Tested with Chi-square

There was no statistically significant difference in $W_{peak}$/kgLBM, $VO_{2peak}$/kgLBM, $HF_{peak}$, $VE_{peak}$, $RER_{peak}$ or $SpO_{2peak}$ during the exercise test between the HC and CF groups.
All patients with CF were non anaemic (Hb 8.4±0.7 mmol/l) and showed neither signs of low grade nor chronic systemic inflammation (serum CRP < 0.5 mg/dl IgG-total 10.8±3.4 g/l [6.6-15.3], see Table 2.

**Table 2.** Exercise results

| Variable | CF (mean ± SD) | Healthy (mean ± SD) | p-value |
|---|---|---|---|
| Rest | | | |
| [PCr] (mM) | 27.5±4.6 | 26.4±3.6 | .620 |
| [Pi] (mM) | 3.3±.5 | 2.9±.5 | .143 |
| [pHi] (mM) | 7.1±.03 | 7.1±0.01 | .844 |
| End Exercise | | | |
| [PCr] (mM) | 11.6±8.7 | 9.4±3.1 | .550 |
| [Pi] (mM) | 19.2±8.2 | 19.7±2.9 | .896 |
| [pHi] (mM) | 6.9±.2 | 6.9±.1 | .512 |
| AU_tHb | .64±.20 | .59±.32 | .778 |
| $HR_{peak}$ (bpm) | 162±12 | 164±9 | .690 |
| $VE_{peak}$ (l·min) | 56.7±14.1 | 55.7±18.7 | .894 |
| $RER_{peak}$ | 0.99±0.07 | 0.97±0.07 | .544 |
| $VO_{2peak}$/kgLBM (ml·min·kg) | 44.6±8.7 | 44.5±8.0 | .968 |
| $W_{peak}$/kgLBM (gramm·kg) | 50.1±9.0 | 49.0±12.7 | .828 |
| Recovery | | | |
| [PCr]_T (sec) | 32.3±3 | 28.8±8.7 | .526 |

PCr = phosphocreatin; Pi = inorganic phosphate; pHi = inorganic pH; AU_tHb = total haemoglobin concentration in arbitrary units (measured with Near Infrared Spectroscopy (NIRS)); $HR_{peak}$ = peak heart rate; $VE_{peak}$ = peak ventilation; $RER_{peak}$ = peak $VCO_2/VO_2$ ratio; $VO_{2peak}$/kgLBM = peak oxygen update per kilogram lean body mass; $W_{peak}$/kgLBM = peak workload per kilogram lean body mass; PCr_T = time constant of phosphocreatine recovery



## $^{31}$P MRS studies

Figure 1 shows a typical series of $^{31}$P MR spectra recorded serially from the quadriceps during the in-magnet maximal exercise test. At a qualitative level, the typical inverse changes in PCr and Pi signal amplitudes at constant ATP amplitudes were observed during the exercise test in all subjects. At the quantitative level, no differences were found between controls and CF patients with respect to bulk changes in quadriceps PCr and Pi content and pH during exercise (Table 2; Figure 2, panels A, B and C, respectively). The change in cytosolic free energy of ATP hydrolysis ($\Delta G_p$) from resting state to maximal workload in quadriceps muscle of the CF patients (described by a sigmoidal relation qualitatively and quantitatively) closely matched the empirical covariation of normalized workload and $\Delta G_p$ in healthy controls (Figure 3). Likewise, the transduction function of [ADP] stimulation of mitochondrial ATP synthesis in quadriceps muscle determined for CF patients matched qualitatively and quantitatively well with matched experimental data on [ADP] and normalized exercise workload in quadriceps muscle of healthy controls

(Figure 4) indicating normal mitochondrial ATP synthetic function. This outcome was confirmed by statistically identical kinetics for PCr resynthesis in quadriceps muscle following exercise for the CF patients and healthy controls (PCr time constant 32.3±3 s versus 28.8±8.7 s, (mean ± SD; CF: n=6 versus HC: n=5, respectively; p = .526). (Fig 5)

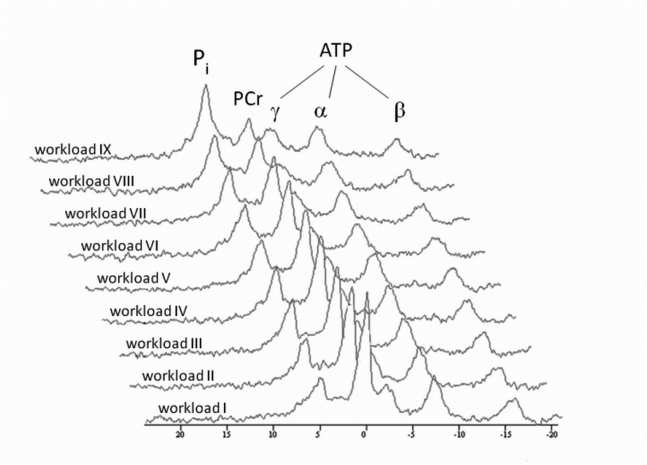


**Fig. 1.** Typical time series of $^{31}$P NMR spectra acquired from the medial head of the quadriceps muscle of the right leg of a study participant performing incremental exercise to exhaustion. Each spectrum corresponds to three summed free induction decays collected during the final 36 s of each 1 minute workload. A 10 Hz line broadening filter was applied prior to Fourier transformation. Pi = inorganic phosphate; PCr = phosphocreatine; ATP = adenosine triphosphate.

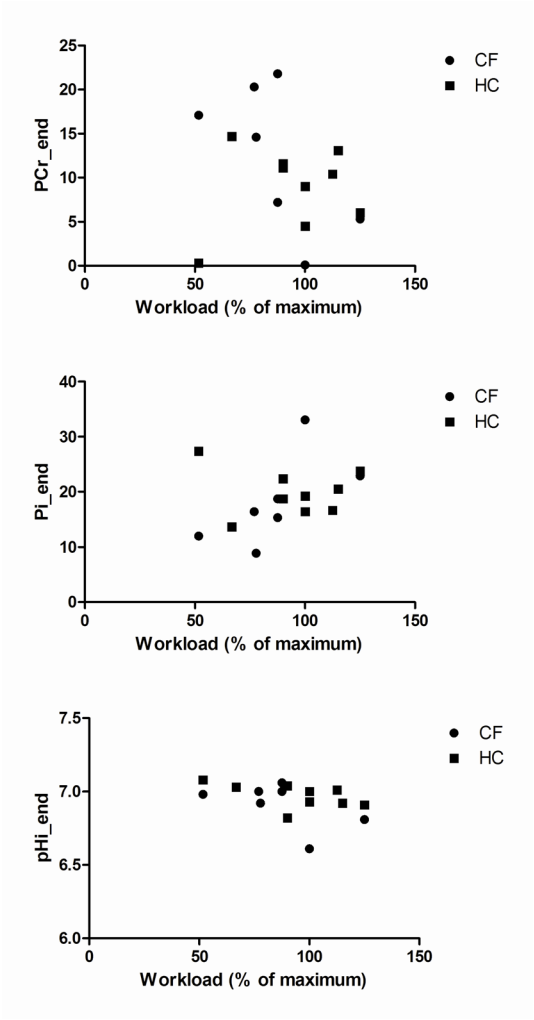


**Fig. 2.** Metabolic endpoints of the in-magnet maximal exercise test in the CF patients (square symbols) and their age- and sex-matched controls (circles).
A: end-PCr concentration (mM); B: end-Pi concentration (mM); C: end-pH. Quadriceps metabolite concentrations and pH were calculated as described in Methods. The specific endpoints are shown as a function of the maximal workload attained in-magnet compared to testing outside the magnet.

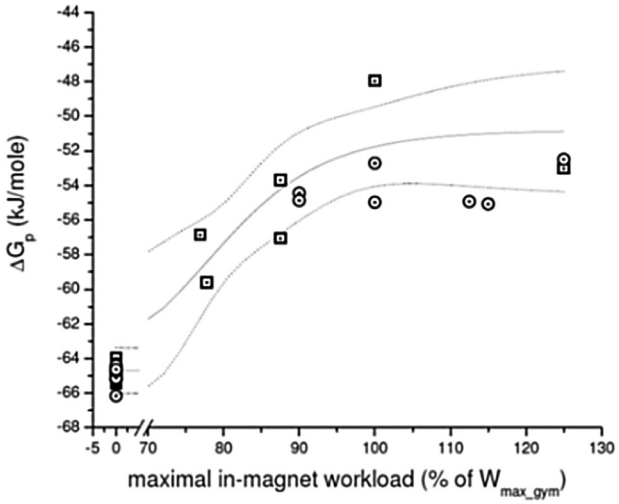


**Fig. 3.** Thermodynamic flow force relation of dynamic bicycling exercise in CF patients (square symbols) and their age- and sex-matched controls (circles).
The solid line shows the fitted sigmoidal Hill relation between g the Gibbs free energy of ATP hydrolysis in quadriceps muscle at rest and during bicycling at maximal in-magnet workload (scaled to the maximal workload attained outside the magnet) for the CF patients. ; dotted lines shows 95% confidence interval of fit. Regression equation: y = -14 / (1 + (X/79)^11) - 51

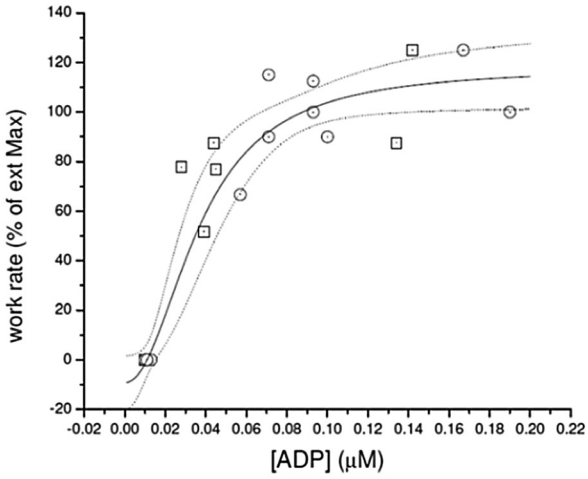


**Fig. 4.** Response function of mitochondrial ATP synthesis to ADP concentration changes in exercising quadriceps muscle of CF patients (square symbols) and their age- and sex-matched controls (circles) as function of scaled in-magnet bicycling workload.
The solid line shows the fitted sigmoidal Hill relation to the control dataset; dotted lines show the 95% confidence interval of the fit. Regression equation: y = 109 * ((X/0.037)^2.1)/(1 + (X/0.037)^2.1) + 118.

## NIRS studies

During NIRS cycling, no statistically significant difference in peak exercise tHb (AU) was found between patients with CF and HC (.64±.20 versus .59±.32; p = .66). (Table 2; Fig. 6)

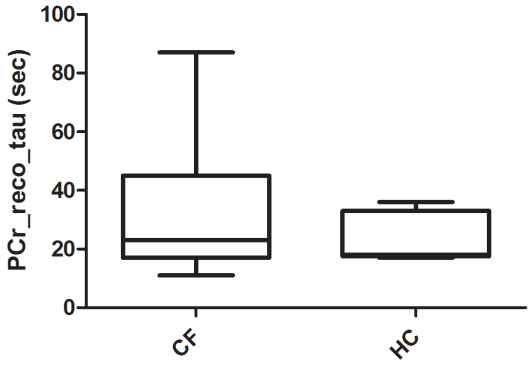


**Fig. 5.** PCr recovery after peak exercise in patients with CF and HC.

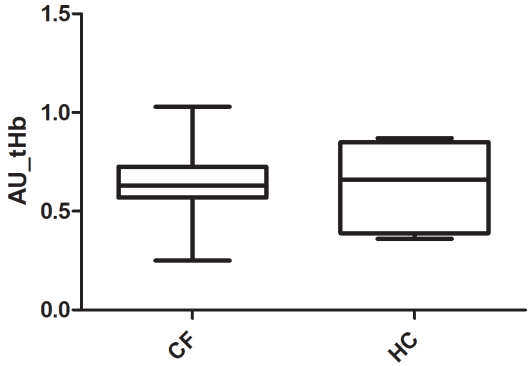


**Fig. 6**. tHb kinetics in patients with CF vs. HC.

## DISCUSSION

This study, in 10 clinically stable adolescents with moderate CF, did not support the hypothesis that exercise capacity in patients with CF is related to impaired skeletal muscle oxidative metabolism due to intrinsic mitochondrial dysfunction.

The studied group (n=10) of adolescents with CF achieved similar peak workloads and $VO_{2peak}$ per kilogram LBM during exercise testing as age- and sex-matched healthy controls (Table 2). Interestingly, an extra post-hoc subgroup analysis based on CFTR mutation Class (I-IV) revealed no differences in exercise capacity ($VO_{2peak}$/kgFFM and $W_{peak}$/kgFFM) between patients from different mutation classes. Unlike Selvadurai et al, who found differences between CFTR mutation classes in 97 participants, our analysis was only based on ten patients with CF all within mutation spectrum Class I-III [24].

The experimental design of the present study was chosen specifically to obtain a highly sensitive platform to test the particular hypothesis under investigation. If present, any limitation of oxidative muscle metabolism in CF, caused by an intrinsic mitochondrial defect or oxygen supply to the muscle, would be readily detected either by altered homeostasis of ATP free energy (Figures 2A, 2B and 3), altered proton balance (Figure 2C), altered kinetics of PCr recovery (qualitatively and quantitatively; Figure 5), or altered Hb saturation during exercise (Figure 6) [25]. In addition, the evaluation of the macroscopic sensitivity of muscle mitochondria to ADP concentration changes (Figure 4) constitutes a robust screening test for any intrinsic mitochondrial defect since ADP is the dominant feedback signal in muscle respiratory control [19]. None of the results of our in vivo $^{31}$P MRS and NIRS measurements in CF muscle during exercise and recovery provided evidence in supporting this hypothesis at any of these levels.

At a first glance, the results of our study appear to be in contrast to the findings of two previous 31P MRS studies of oxidative metabolism in skeletal muscle of CF patients [5, 8]. However, we think the evidence for an intrinsic mitochondrial defect in CF provided by these two studies was weak. De Meer and co-workers did not rule out that their finding of an altered relation between work rate and cytosolic free energy of ATP hydrolysis in exercising forearm muscle could simply be the result of altered contractile economics of the muscle [8]. Such a change in contractile economics of skeletal muscle may stem from a slow-to-fast change in muscle fiber type composition documented, for example, found in patients with heart failure [26]. Secondly, Wells and co-workers did not find any indication for mitochondrial dysfunction in CF muscle in two out of three different exercise experiments they conducted in CF patients. Specifically, they observed identical, normal values for the half-time of PCr recovery (HT) after a single 30 s exercise bout at maximal intensity and repetitive 30 s bouts of exercise at moderate intensity (65% of maximal; [5]). A counterpoint on the present study is that we studied a relatively small group of ten patients with CF and ten HC, which might have implications for comparisons made with the Wells study.

Based on these considerations, the outcome of the present study may help settle the debate on any significant role for impaired oxidative metabolism as a cause of decreased exercise tolerance in CF; at least in the particular patient population of the present study. Recent studies found that hypoxia and/or hypercapnia, often present in moderate to severe patients with CF, could inhibit the mitochondrial oxidative phosphorylation [27, 28]. Additionally, systemic inflammation, which is present in many chronic diseases, is suggested to inhibit mitochondrial function [5], and has been demonstrated to affect exercise capacity [4], and diaphragm contractile force [3] in CF patients. Furthermore, no evidence is available concerning the effect of exercise training on the skeletal muscle metabolism in patients with CF with more severe disease and more systemic inflammation [29]. These mechanisms do not seem to apply to the particular group of non-cachexic, non-hypoxic, non-anaemic CF patients with no presence of systemic inflammation and almost normal lung function that were enrolled in the present study. Conversely, future research may focus on the possible presence of a mitochondrial dysfunction in patients with different CF mutations and/or in patients with a more progressive disease state in the presence of hypoxia, hypercapnia and systemic inflammation.

Despite being less active in the vigorous activity domain, in concert with their normal oxidative metabolism and oxygenation, our patients with CF were capable of achieving workloads and $VO_{2peak}$ per kilogram LBM comparable to healthy controls. In that way, there seems to be no muscular energetic rationale for the previously reported less vigorous daily activity level in patients with CF versus healthy controls [30].

In conclusion, the results of this study provide evidence that patients with CF in a stable clinical status can be trained regularly and that there seems to be no metabolic constraint in this category of patient with CF to benefit from exercise training.

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PART 3 EXERCISE TRAINING

# Chapter 7

# Inspiratory muscle training prior to general exercise training in patients with cystic fibrosis

## a double-blind, randomized controlled trial

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Submitted

## ABSTRACT

*Background*: Benefits of inspiratory muscle training (IMT) on work of breathing in patients with cystic fibrosis (CF) are supported by weak evidence. The impact of IMT on (sub-maximal) exercise capacity and/or trainability is even more inconsistent. The aims of this research were to study the effect of [1] short-term, home-based IMT on work of breathing; [2] short-term, home-based peripheral muscle training program (5BX) on exercise capacity; and [3] IMT on general trainability in patients with CF. *Methods*: A double-blind, randomized controlled trial in patients with CF. Patients were randomized into two groups: [1] six weeks home-based IMT (TG) or [2] placebo IMT (CON). Afterwards, both groups performed the same six weeks home-based 5BX training program, which was followed by a six weeks wash-out period. During follow-up, work of breathing ($T_{T01}$) and exercise capacity ($W_{peak}$/kg; $VO_{2peak}$/kg and $T_{lim}$) were measured. *Results*: Fifty patients were enrolled. There were no significant changes between TG and CON on $T_{T01}$ (p=.08), $W_{peak}$/kg, $VO_{2peak}$/kg (p= .55-.99) and $T_{lim}$ after IMT. No significantly different effects of 5BX between TG and CON were found on exercise capacity ($W_{peak}$/kg (p=.60), $VO_{2peak}$/kg (p=.75), $T_{lim}$ (p=.30)) or $T_{T01}$ (p=.193). Within groups, we found a significant increase in the $T_{T01}$ in the CON (p=.03) and a significant increase in $PI_{max}$ in the TG (p=.005) after IMT. *Conclusions*: Six weeks, home-based, non-supervised IMT did not significantly influence work of breathing compared to controls in patients with moderate CF. Six weeks, non-supervised, 5BX training was insufficient effective to increase exercise capacity ($VO_{2peak}$, $W_{peak}$/kg and $T_{lim}$) in mild-moderate patients with CF. This study can not answer the question whether IMT has a preconditioning effect on general exercise training.

*Trial Registry*: Dutch Trial Register; NTR 2092; URL: www.trialregister.nl

## INTRODUCTION

Cystic Fibrosis (CF) is one of the most common hereditary diseases that is caused by mutations in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR).[1] The clinical manifestations caused by the gene mutation affect the pancreas, bowels, reproductive tracts and, above all, the respiratory system.[1] Furthermore, patients with CF are reported with decreased exercise capacity in which, besides cardiac and local skeletal muscle factors, the ventilatory system can be a limiting factor.[2] First, the continuous airflow obstruction in the lungs makes the patients more prone to (dynamic) hyperinflation, putting the inspiratory muscles in a less efficient, shortened position. [3] Secondly, the hyperinflation position of the thorax increases the (elastic) load of the thorax during inspiration. Both mechanisms increase the load on the inspiratory muscles resulting in an increased work of breathing.[3] The work of breathing has been shown to be increased in rest and during (sub maximal) exercise in patients with moderate CF and,[4] recently, even in patients with mild lung disease.[5] Consequently, the work of breathing increases proportionally with the progression of the disease states.[6]

Current literature suggests that the strength of the inspiratory muscle of patients with CF is sometimes not sufficiently powerful to overcome the additional (elastic) load of the thorax,[7] which explains the negative association between maximal inspiratory muscle strength and work of breathing in patients with CF.[4, 5] This mechanism suggests that reduced inspiratory muscle capacity might place the inspiratory muscles at increased risk of respiratory muscle fatigue,[5] especially when the load on these muscles is progressively increased during (acute) disease progression.[3] In addition, when the respiratory muscles fatigue, the leg blood flow during maximal exercise was reported to decrease.[8] The latter is hypothesized to be induced by reflex vasoconstriction of the blood flow through the locomotor muscles.[9] This 'steal effect' might hamper exercise capacity and trainability in conditions with increased work of breathing.

To increase maximal inspiratory muscle strength, several studies in patients with CF have focused on the effects of inspiratory muscle training (IMT). However, benefits are supported by weak evidence.[10, 11] The knowledge of impact of IMT in patients with CF on (sub-maximal) exercise capacity remains even more inconsistent,[10, 11] especially when considered as an additive to general exercise training.[12] Inspiratory muscle training has been shown to attenuate the respiratory metabo-reflex during fatiguing breathing tasks in healthy participants.[13] This suggests that improving inspiratory muscle capacity might decrease work of breathing during exercise, making the inspiratory muscles more resistant to fatigue, which in turn might improve exercise capacity or trainability of the locomotor skeletal muscles. Therefore, the hypothesis of our study was that (1) a six-week home-based IMT program decreases work of breathing and (2) a six-week home-based

peripheral muscle training program increases exercise capacity in patients with CF, and (3) IMT increases general trainability in patients with CF.

## METHODS

### Patients and Intervention

Patients were informed about the study by HH, MW, CE or HA. Patients were enrolled by MW. Patients were enrolled in the study between August 2009 and February 2013. Inclusion criteria were: CF patients > 11 years of age, free from fever, without presence of recent pulmonary or gastrointestinal exacerbation, without contra-indications for maximal exercise testing [14] and with sufficient knowledge of the Dutch spoken and written language and not being included in another study. The research protocol was approved by the medical ethics committee of the University Medical Center Utrecht. The trial was conducted in accordance to the declaration of Helsinki and good clinical practice rules and recoded in the Dutch Trial Register with identification code NTR 2092. All patients, and (when < 18 years of age) their parents gave written informed consent for participation in the study. After inclusion, patients were randomly allocated to the training group (performing inspiratory muscle training (TG)) or control-placebo group (performing sham IMT (CON)). Randomization was performed using a computer-generated random number program. Allocation of the numbers to included patients was based on order of inclusion. Both the primary researcher (MW) and the patients were blinded for group allocation. Patients were instructed to avoid speaking about the intervention at the post-IMT measurement. All measurements, except for flow-volume curves in the lung function section, were performed by MW. The key to the patient codes was administered by HH.
Both groups performed a six weeks home-based IMT with the TG starting on 30% of $P_{Imax}$ and the CON started and stabilized at 10% of $P_{Imax}$, using a threshold loading device (Threshold IMT, Respironics, New Jersey, USA). Both groups performed four 2-minute intervals IMT divided by 1-minute pause breaks, once a day and five days a week. Only the TG patients were encouraged to increase the training load with 5% when the rate of perceived exertion, measured with the OMNI scale,[15] was equal to or below 5. The IMT protocol was instructed by HH. Patients were instructed to record their daily training in a logbook.
After the six weeks IMT period, all participants executed the same standardized home-based exercise program for six weeks. The program consisted of five basic exercises (5BX) that had to be performed within 11 minutes for five days a week.[16] Starting level was based on the baseline 5BX exercise test. Patients were instructed by MW how to perform

the exercises at the start of the study. Training intensity progressed to a higher level when individual participants were able to perform the five basic exercises within 11 minutes. Participants were instructed to record the training level in a logbook.

### Measurements

All measurements were performed before and after the six-weeks IMT program, the six–weeks 5BX program, and after a 6 weeks follow-up. Individual data were collected over the course of one visit. Patients were asked to avoid heavy meals and strenuous exercise from the evening before their testing session. All measurements were performed at the exercise laboratory of the Child Development and Exercise Center, University Medical Center Utrecht, the Netherlands.

### Anthropometry

Anthropometric values, including weight and height were measured using an electronic scale (Seca, Birmingham, UK) and a stadiometer (Ulmer stadiometer, Prof. E. Heinze, Ulm, Germany), respectively. Body mass index (BMI) was calculated by dividing weight by squared height.

### Lung function

Lung function was measured using the ZAN Betterflow (ZAN 100, Accuramed BVBA, Lummen, Belgium). The work of breathing during quietly breathing was measured with the non-invasive Tension Time Index ($T_{T0.1}$) calculated according to the following formula: $T_{T0.1} = P_{01mean} / P_{Imax} \times T_I/T_{TOT}$,[6] where $P_{01}$ the mouth occlusion pressure ($cmH_2O$), $PI_{max}$ the maximum inspiratory pressure ($cmH_2O$) and $T_i/T_{tot}$ the respiratory duty cycle reflected by the ratio between the inspiratory time ($T_i$) and the total duration of the breathing cycle ($T_{tot}$). $P_{01mean}$ is the mean inspiratory pressure, calculated by $5*P_{0.1}*Ti$. All variables are measured in resting, sitting, position conform current used protocols.[17] $PI_{max}$ was measured with the Micro-RPM, Viasys, Houten, The Netherlands. $P_{0.1}$ ($cmH_2O$) was measured with the ZAN Betterflow (ZAN 100), Accuramed BVBA, Lummen, Belgium.

### Exercise capacity

Exercise capacity was assessed using a cardiopulmonary exercise test (CPET) according to the Godfrey protocol (*Godfrey et al, 1974*).[18] The Godfrey protocol was performed on an electronically braked cycle ergometer (Lode Corrival, Procare BV, Groningen, The

Netherlands). Participants began with unloaded cycling and the workload increased every minute in a fixed interval based on height (10 W/min <120 cm; 15 W/min 120-150 cm; 20 W/min > 150cm), independent of sex, until the patient stopped due to volitional exhaustion. Throughout the test, adolescents breathed into a mask connected to a calibrated metabolic cart (ZAN 600, Accuramed BVBA, Lummen, Belgium). Expired gas passed through a flow meter, oxygen analyzer, and a carbon dioxide analyzer. The flow meter and gas analyzer were connected to a computer, which calculated breath-by-breath minute ventilation (VE), oxygen uptake ($VO_2$) carbon dioxide production ($VCO_2$), and respiratory exchange ratio (RER) from conventional equations. Heart rate (HR) was also monitored continuously by a 12-lead electrocardiogram (Cardioperfect, Accuramed BVBA, Lummen, Belgium). Transcutaneous oxygen saturation ($SpO_2$%) was measured with a pulse oximeter placed on the index finger (Massimo Rad 8, Tilburg, The Netherlands). Peak exercise parameters ($VO_{2peak}$, $W_{peak}$) were defined as the mean values achieved during the final 30 seconds of the test. For the definition of maximal effort both subjective and objective criteria were used. Subjective criteria are: "unsteady biking," "sweating," "facial flushing," and "clear unwillingness to continue despite encouragement".[19] The objective criteria are: HR > 180 bpm and/or RER > 1.00 [19] and/or breathing reserve (1-$VE_{peak}$/MVV) < 0.30. Subjects had to meet the subjective criteria and at least one of the objective criteria's to validate that the participant had performed maximal effort. Exercise data of participants who did not meet the criteria for a maximal effort were not included in the analysis of exercise parameters.

## Endurance test

After a 10 minutes pause break, patients performed a continuous work rate test (CWRT) to assess volitional endurance time ($T_{lim}$) on cycling at 75% of attained $W_{peak}$ of the baseline CPET.[20] Patients who did not perform a maximal effort at the baseline CPET were not included in the (CWR) analysis during follow-up.

## Data Analysis

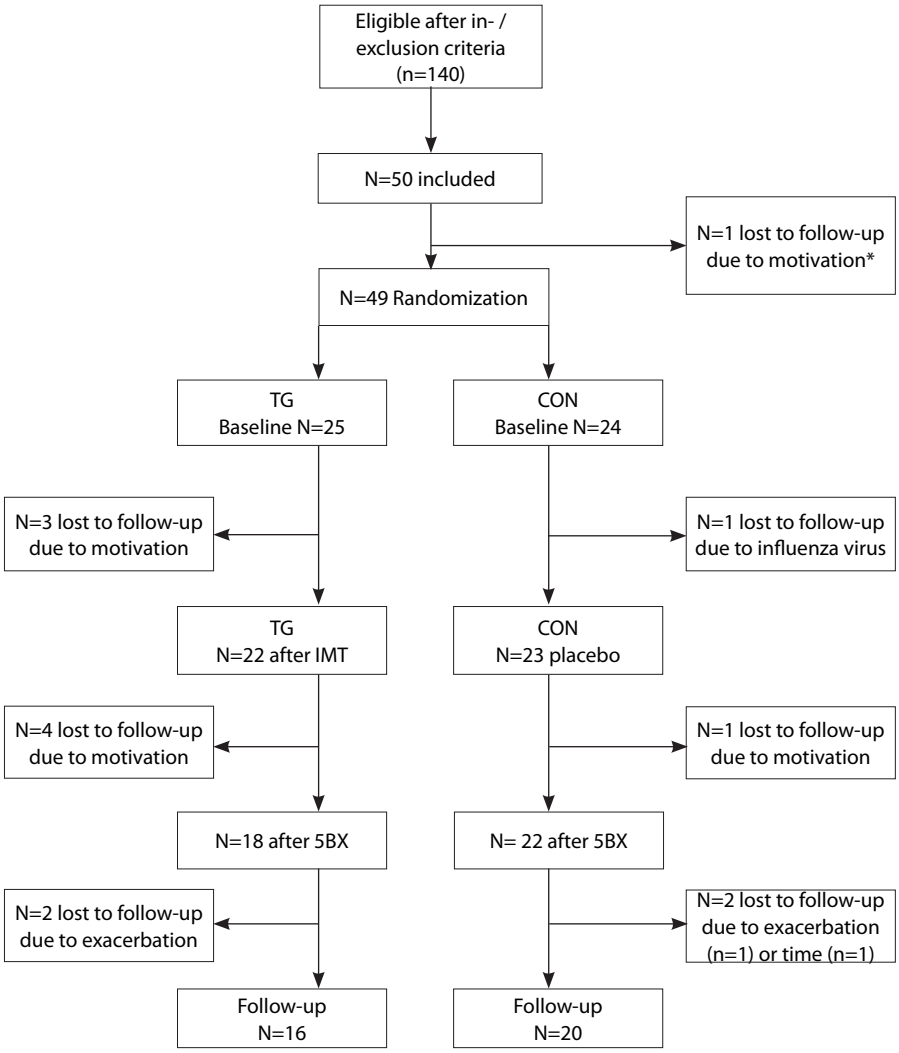
To analyze the data, the Statistical Package for the Social Sciences (SPSS, version 17.0; SPSS Inc., Chicago, IL, USA] was used. Normality of the data was checked using the Kolmogorov-Smirnov and Shaprio-Wilk test. A two-factor (intervention, time) repeated-measures ANOVA was used to analyze between group effects over time within the different time-intervals. Within-group comparisons were made with paired-sample t-tests (parametric) or Wilcoxon Signed Rank tests (non-parametric). Statistical significance was set at .05. Data are shown as mean ± standard deviation.

The desired power was set at 0.90. Out of the database of our laboratory a mean $W_{peak}$ of 143.7±42.8 watt in adolescents with CF was calculated. Based on clinical expert consensus in our exercise laboratory, a change of 20% in $W_{peak}$ was considered clinical relevant (conservative calculation), meaning an increase of 28.7. With our objective to include 60 patients (30 patients each arm), we can detect a significant change of 25.3 Watt ($30=(1.96+1.28)^2 / (143.7-X)^2 \rightarrow 25.3$) A clinical relevant difference would thereby also reach statistical significance in a complete case analysis.
Analyses were performed on intention-to-treat basis as well as per protocol. With the intention-to-treat analysis all patients were analyzed as randomized, including the ones who did not train as instructed, disregarding missing data. Per protocol analyses were performed with the data of patients who trained as intended.

## RESULTS

### Patients and follow up

Fifty patients (35%) with CF, aged 12-37 years (23 males and 27 females), of the initially invited 140 patients matching the in/exclusion criteria, were included in the study. Despite extensive efforts (expanding the inclusion to age > 18 years and including three patients from the University Hospital Maastricht), we were not able to include the intended 60 patients. Figure 1 gives information about sample size during follow-up.
One participant dropped out during the pre-baseline period, which was initially included in the study. After randomization to the training and control group, 49 patients completed the baseline measurements before IMT. Baseline characteristics did not differ between the two groups (see Table 1). Moreover, the main clinical characteristics (CFTR mutation classes; colonization with Pseudomonas Aeruginosa; presence of pancreatic insufficiency / cystic fibrosis related diabetes (CFRD)) were comparable. Three patients, who were both assigned to the TG, did not complete the IMT training period, due to motivational issues. Screening the training diaries for IMT and 5BX, we could identify two patients in the IMT TG who did not perform IMT as intended and one patient who did not perform 5BX as intended. These patients were left out in per protocol analyses. All data, except for age, were, according to the Kolmogorov-Smirnov test, from a normally distributed population.




*Flow (n) of numbers for analyzed main outcome measures for intention-to-treat analysis*

| | TG | | | | CON | | | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | IMT | 5BX | Follow-up | Baseline | IMT | 5BX | Follow-up |
| $FEV_1$ | 22 | 22 | 18 | 16 | 23 | 23 | 22 | 20 |
| $T_{T01}$ | 19 | 19 | 13 | 13 | 20 | 20 | 17 | 16 |
| $P_{01}$ | 20 | 20 | 14 | 14 | 20 | 20 | 21 | 19 |
| $PI_{max}$ | 21 | 21 | 15 | 15 | 22 | 22 | 17 | 16 |
| $VO_{2peak}$/kg | 18 | 18 | 12 | 9 | 20 | 20 | 17 | 15 |
| $W_{peak}$/kg | 19 | 19 | 14 | 11 | 22 | 22 | 19 | 17 |
| $T_{lim}$ | 15 | 15 | 13 | 9 | 18 | 18 | 16 | 13 |

**Fig. 1.** Flow diagram with flow of individual measured data through study.
*Initial period of study patients were followed during a 6 week baseline period

**Table 1.** Baseline characteristics

| | TG (n= 25) | CON (n= 24) | p |
|---|---|---|---|
| Gender (male/female) | *14/11* | 13/11 | 0.898[a] |
| Age (years) | 18.7±7.7 (12.2-37.7) | 16.1±4.9 (12.1-29.3) | 0.222[a] |
| Height (cm) | 168.8±12 | 164.0±13 | 0.191[b] |
| Weight (kg) | 57.2±12.9 | 52.0±12.6 | 0.158[b] |
| BMI ($kg*m^{-2}$) | 19.8±2.2 | 19.0±2.0 | 0.202[b] |
| CF Classification [21] | ΔF508 homozygote n=18<br>ΔF508 heterozygote n= 4<br>other n=1; unknown n=2<br>(severe* n=20; mild** n=3; unknown n=2) | ΔF508 homozygote n=11<br>ΔF508 heterozygote n=11<br>other n=1; unknown n=1<br>(severe n=18; mild n=4; unknown n=2) | 0.989[a] |
| Pancreatic insufficient | n=6 | n=5 | .859[c] |
| Chronic PA colonization[24] | never colonized n=3<br>all cultures PA negative n=9<br>intermittent n=4<br>chronic n=9 | never colonized n=3<br>all cultures PA negative n=9<br>intermittent n=5<br>chronic n=6<br>unknown n=1 | 0.834[a] |
| $FEV_1$ ($\%_{pred}$) (L) | 76±26 (2.78±1.16) | 78±16 (2.64±0.81) | 0.745[b] |
| RV/TLC (%) | 33.29±12.54 | 29.26±8.99 | 0.255[b] |

[a] Mann-Whitney U-test, values are presented as mean ± SD (range); [b] Independent sample t-test, values are presented as mean ±SD; [c] Chi-square test; * CF classes 1, 2 and 3; ** CF classes 4 and 5; BMI, body mass index; $FEV_1$, forced expiratory volume in 1 sec; RV/TLC, ratio residual volume/total lung capacity;

## IMT training period

Using intention-to-treat, no significant changes were found for $T_{T01}$ (p=.08), $PI_{max}$ (p=.22), $T_i/T_{tot}$ (p=.57), $P_{0.1}$ (p=.07) and $P_{01mean}/PI_{max}$ (p=.21) between TG and CON. Within groups, we found a stable $T_{T01}$ in the TG (p=1.0), and a significant increase in the CON (p=.03). $PI_{max}$ increased significantly in the TG (p=.005). The duty cycle ($T_I/T_{TOT}$) remained unchanged within both groups (TG: p = 1.0; CON: p = 0.39). Furthermore, no significant changes were found for $VO_{2peak}$/kg (p=.99), $W_{peak}$/kg (p=.51) or $T_{lim}$ (p=.88) between TG and CON. (Table 2) Furthermore, no significant within group differences were found for exercise parameters in both groups ($W_{peak}$/kg: CON p= .52; TG p=.83, $VO_{2peak}$/kg: CON p=.21; TG p=.51, and $T_{lim}$: CON p=.95; TG p=.91). (Table 2)

Per protocol, no significant change between TG and CON was found for ventilatory data ($T_{T01}$ (p=.083), $PI_{max}$ (p=.152), $T_i/T_{tot}$ (p=.720), $P_{0.1}$ (p=.067) and $P_{01mean}/PI_{max}$ (p=.238)). Within groups, we found a significant increase in $PI_{max}$ in the TG (p=.002).

No significant per protocol changes were found for $VO_{2peak}$/kg (p=.479), $W_{peak}$/kg (p=.334) or $T_{lim}$ (p=.715) between TG and CON. (Table 2) Furthermore, no significant within group differences were found for exercise parameters in the TG ($W_{peak}$/kg: p=.444, $VO_{2peak}$/kg: p=.937, and $T_{lim}$: p=.609).

## 5BX training period

In exercise capacity parameters, no significant intention-to-treat changes over time were found for 5BX on $W_{peak}$/kg (p=.60), $VO_{2peak}$/kg (p=.75) or $T_{lim}$ (p=.30) between TG and CON. There were no within group differences for $W_{peak}$ (CON p= .95; TG p= .32), $W_{peak}$/kg (CON p= .80; TG p= .27), $VO_{2peak}$ (CON p= .95; TG p= .88) and $VO_{2peak}$/kg (CON p= .81; TG p= 1.0). (Table 2) We found no significant changes over time for 5BX on $T_{T01}$ (p=.193) between TG and CON. No significant pre-post differences within groups for $FEV_{1\%pred}$ (CON p=.59; TG p=.94) were noted.

Per protocol, no significant changes over time were found for $W_{peak}$/kg (p=.794), $VO_{2peak}$/kg (p=.760) or $T_{lim}$ (p=.989) between TG and CON. No within group differences were found the $W_{peak}$/kg (TG p=.952; CON p=.586), $VO_{2peak}$/kg (TG p=.813; CON p=.469) or $T_{lim}$ (TG p=.500; CON p=.432). Furthermore, no significant per protocol changes over time were found for $P_{0.1}$ (p=.257), $T_{T01}$ (p=.185), $T_i/T_{tot}$ (p=.227), $P_{0.1}/PI_{max}$ (p=.122), $PI_{max}$ (p=.674) or $FEV_{1\%pred}$ (p=.834) between TG and CON. Per protocol within group analysis revealed no significant differences for any ventilatory parameter in the TG (p = .229 - .724) or CON (p=.240-.832).

**Table 2.** Intention-to-treat results by group during follow-up

| | Group | Baseline | T=1 Post-IMT | $\eta_p^2$ | T=2 Post-5BX | $\eta_p^2$ | Follow-up | $\eta_p^2$ |
|---|---|---|---|---|---|---|---|---|
| *Ventilation* | | | | | | | | |
| $FEV_{1\%pred}$ (L/min) | TG<br>CON | 76±26<br>78±17 | 73±25<br>80±18 | 0.085 | 75±27<br>80±18 | .003 | 78±26<br>81±17 | .016 |
| $PI_{max}$ | TG<br>CON | 96±25<br>106±26 | 106±26<br>109±27 | .037 | 111±26<br>110±21 | .003 | 106±27<br>105±20 | .003 |
| $P_{01}$ | TG<br>CON | 2.9±2.0<br>2.9±1.5 | 3.1±2.3<br>4.2±2.6 | .083 | 3.0±1.4<br>4.0±2.3 | .035 | 3.2±1.6<br>3.7±1.6 | .008 |
| $P_{01mean}/PI_{max}$ | TG<br>CON | 0.20±0.12<br>0.21±0.12 | 0.20±0.13<br>0.27±0.17 | .041 | 0.20±0.09<br>0.22±0.12 | .078 | 0.18±0.09<br>0.23±0.12 | .055 |
| $T_i/T_{tot}$ | TG<br>CON | 0.43±0.06<br>0.44±0.06 | 0.43±0.07<br>0.44±0.05 | .008 | 0.40±0.06<br>0.44±0.05 | .047 | 0.43±0.04<br>0.44±0.05 | .022 |
| $T_{T01}$ | TG<br>CON | 0.085±0.053<br>0.089±0.044 | 0.082±0.041<br>0.114±0.068 | .080 | 0.078±0.03<br>0.096±0.049 | .058 | 0.077±0.036<br>0.101±0.044 | .013 |
| Exercise | | | | | | | | |
| $VO_{2peak}$/kg | TG<br>CON | 35.30±8.66<br>47.74±7.76 | 37.14±7.86<br>42.75±8.63 | .000 | 37.5±6.9<br>43.43±9.56 | .004 | 38.2±7.9<br>42.48±7.77 | .143 |
| $W_{peak}$/kg | TG<br>CON | 3.0±0.7<br>3.4±0.7 | 3.0±0.7<br>3.6±0.6 | .011 | 3.1±0.7<br>3.5±0.6 | .009 | 3.0±0.7<br>3.5±0.7 | .096 |
| $T_{lim}$ | TG<br>CON | 325±150<br>384±145 | 340±256<br>360±154 | .001 | 338±123<br>406±149 | .040 | 289±167<br>433±198 | .007 |

Values are presented as mean ± SD; $PI_{max}$, maximum inspiratory pressure; $P_{01}$, mouth occlusion pressure; $P_{01mean}$ mean inspiratory pressure; $T_i/T_{TOT}$, respiratory duty cycle; $P_{0.1mean}/PI_{max}$, ratio between mouth occlusion pressure and maximal inspiratory pressure; $T_{T0.1}$, non-invasive tension time index; TG, training group; CON, control group.

**Table 3.** Per protocol results by group during follow-up

| | Group | Baseline | T=1 Post-IMT | $\eta_p^2$ | T=2 Post-5BX | $\eta_p^2$ | Follow-up | $\eta_p^2$ |
|---|---|---|---|---|---|---|---|---|
| *Ventilation* | | | | | | | | |
| $PI_{max}$ | TG<br>CON | 98±24<br>105±28 | 108±25<br>109±27 | .051 | 113±27<br>111±23 | 0.006 | 110±27<br>107±20 | 0.006 |
| $P_{01}$ | TG<br>CON | 3.2±2.1<br>3.0±1.5 | 3.2±2.4<br>4.3±2.7 | .086 | 3.0±1.4<br>4.0±2.3 | 0.040 | 3.1±1.7<br>3.7±1.6 | 0.013 |
| $P_{01mean}/PI_{max}$ | TG<br>CON | 0.20±0.10<br>0.21±0.12 | 0.21±0.14<br>0.27±0.17 | .037 | 0.20±0.09<br>0.22±0.12 | 0.083 | 0.18±0.09<br>0.21±0.08 | 0.045 |
| $T_i/T_{tot}$ | TG<br>CON | 0.43±0.06<br>0.45±0.06 | 0.43±0.07<br>0.44±0.05 | .003 | 0.40±0.06<br>0.44±0.06 | 0.045 | 0.42±0.04<br>0.43±0.05 | 0.033 |
| $T_{T01}$ | TG<br>CON | 0.085±0.041<br>0.093±0.044 | 0.084±0.042<br>0.116±0.070 | .079 | 0.078±0.033<br>0.097±0.050 | 0.062 | 0.074±0.034<br>0.094±0.035 | 0.008 |
| Exercise | | | | | | | | |
| $VO_{2peak}$/kg | TG<br>CON | 36.72±7.77<br>42.04±7.51 | 36.83±8.71<br>43.38±7.21 | 0.015 | 37.52±6.91<br>44.45±8.86 | 0.004 | 39.73±8.59<br>44.95±5.67 | 0.138 |
| $W_{peak}$/kg | TG<br>CON | 3.1±0.7<br>3.5±0.6 | 3.1±0.8<br>3.6±0.6 | 0.026 | 3.1±0.73<br>3.6±0.6 | 0.002 | 3.2±0.7<br>3.6±0.6 | 0.046 |
| $T_{lim}$ | TG<br>CON | 301±134<br>377±148 | 278±146<br>375±149 | 0.004 | 304±131<br>396±153 | 0.000 | 282±170<br>467±229 | 0.026 |

Values are presented as mean ± SD; $PI_{max}$, maximum inspiratory pressure; $P_{01}$, mouth occlusion pressure; $P_{01mean}$ mean inspiratory pressure; $T_I/T_{TOT}$, respiratory duty cycle; $P_{0.1mean}/PI_{max}$, ratio between mouth occlusion pressure and maximal inspiratory pressure; $T_{T0.1}$, non-invasive tension time index; TG, training group; CON, control group.

## Follow-up

All the ventilatory and exercise parameters remained stable during the intention-to-treat follow-up period. With intention-to-treat, no significant changes were found for $PI_{max}$ (p=.753), $P_{01}$ (p=.623), Ti/Ttot (p=.410) or $T_{T01}$ (p=.666) between TG and CON. For exercise parameters, we found no significant changes for $W_{peak}$/kg (p=.108), $VO_{2peak}$/kg (p=.068) or $T_{lim}$ (p=.719) between TG and CON.

Per protocol, no significant between group effects over time were found for $PI_{max}$ (p=.687), $P_{01}$ (p=.539), Ti/Ttot (p=.318) or $T_{T01}$ (p=.657). For exercise parameters, we found no significant between group effects over time on $W_{peak}$/kg (p=.295), $VO_{2peak}$/kg (p=.081) or $T_{lim}$ (p=.430).

## DISCUSSION

The hypothesis of our study was that (1) a six-week home-based IMT program decreases work of breathing and (2) a six-week home-based peripheral muscle training program increases exercise capacity in patients with CF, and (3) IMT increases general trainability in patients with CF.

We found no changes in work of breathing between TG and CON after six weeks IMT in mild-moderate patients with CF. Furthermore, a home-based six-week peripheral muscle training program did not improve exercise capacity in clinically stable patients with CF. So this study can not answer the question if IMT has a preconditioning effect on general exercise training.

Despite the absence of a significant change over time between TG and CON, within groups the work of breathing ($T_{T01}$) showed a stable trend in the TG and a significant increase in the CONs after IMT, which can be explained by a significantly increased $P_{01}$. The stable trend for $T_{T01}$ with IMT versus the increasing trend in the CONs might be of clinical value. Lower work of breathing demonstrates a higher efficiency of the respiratory muscles and is associated with a decreased risk of respiratory muscle fatigue.[22]

Current literature reports higher mouth occlusion pressure ($P_{0.1}$) values in patients with CF compared to healthy controls.[4, 23] In patients with COPD, it has been suggested that the respiratory neural drive (which is reflected by the $P_{0.1}$) increases as a strategy to maintain effective ventilation when respiratory muscles become weaker.[24] Our study shows that $P_{0.1}$ did not change with IMT in patients with CF, but that it increased in the CONs. To our knowledge this has not been reported before in patients with CF.

The effect of IMT on $PI_{max}$ in CF is widely studied. The evidence for an effect on $PI_{max}$ is still weak,[10, 11] while there is a lack of evidence that describes the effect of IMT on the other components of work of breathing. In this study, we found no significant effect of IMT on $PI_{max}$ between TG and CON, which can be explained by a small increase in $PI_{max}$ in CON (+4 cmH2O; p=.24) and a moderate, but significant, increase in the TG (+9 cmH2O; p<.01). Like the study from de Jong et al with comparable patient characteristics, we found the difference in intensity of IMT between CON (stable at 10% $PI_{max}$) and TG (volitional increments after starting at 30% $PI_{max}$) to be relatively small, which could have attenuated the IMT training effect.[25] Greater IMT training effects have been found in studies with more intensive, supervised and longer duration training strategies (40-80% of $PI_{max}$).[26, 12] Probably higher training intensities lead to greater increases in $PI_{max}$. A recent study in rodents showed that a higher training load was more effective in altering muscle fiber cross-sectional area of the diaphragm than extended duration sessions.[27]

In line with former studies, low-moderate intensity IMT had no effect on exercise capacity in this group of patients with CF.[10, 11] The only study that showed a direct effect of IMT on exercise performance was performed with a high intensity (80% $PI_{max}$), with no

effect in the 20% $PI_{max}$ training group.[26] Based on experiences in healthy participants, resistive loads of at least 60% of $PI_{max}$ are necessary to improve exercise capacity.[28] For pragmatic reasons, we chose to start at an intensity of 30% of $PI_{max}$ at baseline with volitional continuous 5% increments based on OMNI score.

Also, due to moderate compliance in recording the training diaries, we were not able to sufficiently verify quality and quantity of the unsupervised training sessions. Nevertheless, from the diaries completed, the mean training load increased to 34% from $PI_{max}$ at baseline, ranging from 30% (no increase of baseline load) to 44% (+14% increase). This mean increase might have been insufficient to create an effective training load.

To our knowledge, the direct effect of IMT alone on $T_{lim}$ during a Continuous Work Rate Test [20] in patients with CF has not been studied before. In contrast to our results, previous studies found a significant increase in exercise time on a cycle ergometer [26] and treadmill [29] respectively. Reason for this might be that exercise time to volitional fatigue at a constant workload shows relatively large coefficients of variation (~27%).[30] Based on the direct effect of IMT in patients with COPD on exercise duration using the same test protocol,[20] we chose for this test in the present study. However, for future research, we suggest to add a more performance based test (i.e. time trial).[30]

There is strong evidence that regular general (peripheral muscle) exercise training has the potential to attenuate the annual decrease in lung function ($FEV_1$) in the short and long term.[31] Literature shows an annual decline of $FEV_1$%pred of 1-3.2% in patients with CF.[32, 33] Although the 5BX program had no significant effect on exercise capacity, with a post-hoc analysis, we found an overall 0.6% increase in $FEV_1$%pred after general exercise training. As $FEV_1$%pred is a significant predictor of 2-5 year survival in patients with CF,[34, 35] this finding is clinically relevant. However, the effect size in our group is smaller than in comparable groups.[12, 36] We assume that the intensity of the 5BX training program was not sufficient to reach optimal training effects due to the short exercise duration and home-based, non-supervised, training regime in our study. For future studies, we suggest the use of longer, supervised and more intensive exercise protocols.

Regardless of the low exercise intensity of the 5BX program, $VO_{2peak}$/kg non-significantly increased with 0.8%, independent of preconditioning with IMT (p=.75; $VO_{2peak}$/kg +3% in TG versus +1.3% in CONs). However, in the light of the reported monthly decline of 0.17 ml/min/kg in children (8-17 years) with CF,[32] at least a short-term (small) increase or stabilization of $VO_{2peak}$ is of significant clinical relevance. Additionally, with the dramatic increase in mortality in patients with CF with a $VO_{2peak}$/kg less than 32 ml/min/kg (*Pianosi et al, 2005*),[32] it would be of relevant prognostic value to improve $VO_{2peak}$ in this subgroup of patients. A post-hoc analysis in this subgroup showed a significant (p=.02) increase in $VO_{2peak}$ from 28.10 ml/min/kg at baseline to 31.19 ml/min/kg after the 5BX regime, with no interaction effect of IMT (p=.686). Covariates as $FEV_{1\%pred}$ <65% or the presence of static

hyperinflation (RV/TLC > 30%) at baseline had no significant influence on the effect size of both training regimes (p=.889 for RV/TLC and p=.667 for $FEV_{1\%pred}$ < 65%).

In conclusion, a six-weeks, home-based, non-supervised IMT program did not significantly decrease work of breathing compared to controls in patients with moderate CF. A six weeks, non-supervised, 5BX training program was insufficient effective to increase exercise capacity in mild-moderate patients with CF. This study can not answer the question if IMT has a preconditioning effect on the 5BX training program.
Further research should focus on the best working exercise protocols (intensity/dose-response relationship/timing) for IMT and/or general exercise training. Furthermore, different regimes (supervised / home-based versus in-hospital) and types (aerobic/anaerobic/combinations) in different (severe; moderate; mild) patients with CF should be explored. This further research needs to be performed to develop better guidelines for different populations regarding age, lung function and diseases status. Furthermore, future research should also focus on the effects of IMT in patients with more pronounced inspiratory muscle weakness.

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# Chapter 8

# Summary, General discussion and Future research directions

Maarten S. Werkman

## SUMMARY

Exercise testing and training are cornerstones in regular CF care. However, no consensus exists in literature about which exercise test protocol should be used for individual patients. Furthermore, divergence exists in insights about both the dominant exercise limiting mechanisms and the possibilities to aim and institute exercise training strategies, based on these individual limitations.
Therefore, this thesis intends to expand current knowledge in [1] alternative exercise test procedures in CF; [2] exercise limiting mechanisms in CF; and [3] exercise training of the inspiratory and skeletal muscles.

In the first part of this research report we describe two different methodologies of exercise testing that were developed in patients with CF and were analyzed for their usefulness. *Chapter 2* described the verification of $VO_{2peak}$ obtained during traditional cardiopulmonary exercise testing (CPET) with a supramaximal exercise procedure. The development and the validity of an individualized exercise test protocol in children and adolescents with CF are described in *Chapter 3*. Subsequently, *Chapter 4* presents an alternative method to estimate $VO_{2peak}$ developed and validated in adolescents with CF.

The major findings of the first part of the thesis:

- The $VO_{2peak}$ measured with traditional CPET seems to reflect the true $VO_{2peak}$ in adolescents with CF (*Chapter 2*),
- $W_{peak}$ can be reliably predicted with standard measured anthropometric variables (*Chapter 3*),
- The predicted $W_{peak}$ can be reliably used as guideline to individualize workload increments during CPET (*Chapter 3*),
- $VO_{2peak}$ can be reliably predicted from $W_{peak}$ obtained with a traditional CPET combined with gender in adolescents with CF without the necessity of direct gas analysis (*Chapter 4*).

The second part of this thesis focuses on two previously described exercise limiting mechanisms in patients with CF. *Chapter 5* presents a study of the possible role of static hyperinflation as an exercise limiting factor, while *Chapter 6* describes a study of the possible role of a CF specific locomotor skeletal muscle dysfunction.

The major findings of the second part of the thesis:

- The presence of static hyperinflation alone does not strongly influence ventilatory limitations during (peak) exercise in adolescents with CF (*Chapter 5*),
- The RV/TLC > 30% criterion for static hyperinflation is a slightly stronger predictor of $W_{peak}$/kg and $VO_{2peak}$/kg than the traditional $FEV_{1\%pred}$ (*Chapter 5*),
- Oxidative exercise metabolism and oxygenation kinetics in clinically stable adolescents with CF with mild lung function impairment and without systemic inflammation seem to be comparable to healthy controls (*Chapter 6*).

The third part of this thesis (Chapter 7) presents a study of the effects of two different types of exercise interventions, i.e. short-term, home-based inspiratory muscle training (IMT) and short-term, home-based peripheral muscle training program in patients with CF and the possible preconditioning effect of inspiratory muscle training prior to general exercise training.

The major findings of this part of the thesis:

- A six-week, home-based, non-supervised IMT program does not significantly decrease work of breathing in patients with mild lung function impairment compared to control patients,
- Six weeks, non-supervised, peripheral muscle training was insufficient effective to increase exercise capacity in mild-moderate patients with CF.

## GENERAL DISCUSSION

### Exercise testing

Peak oxygen uptake ($VO_{2peak}$) is a strong predictor of mortality and a widely used outcome measure in exercise intervention studies in patients with CF. Therefore the accuracy of its assessment is of clinical importance.[1] Preliminary cessation of the exercise test by the patient before the attainment of cardiac or respiratory limits, may underestimate the real $VO_{2peak}$. Furthermore, the preliminary termination seen in small children and adolescents may be due to workload increments that are too large, resulting in premature exhaustion of the muscles of the lower limbs before attaining cardiac or respiratory limits.[2] We confirmed the accuracy of the $VO_{2peak}$ measured with direct gas analysis using the Godfrey protocol with supramaximal verification independent of the attainment of maximal effort. Saynor et al. [1] found no statistically significant difference in children and adolescents with CF between $VO_{2peak}$ attained during traditional incremental ramp exercise and during

a supramaximal (constant cycling at 110% of $W_{peak}$) verification protocol. Interestingly, tests using secondary criteria to define maximal effort as peak heart rate ($HR_{peak}$) > 180 bpm; $HR_{peak}$ > 95% of age predicted $HR_{peak}$; RER > 1.00 and RER > 1.10 showed an underestimated $VO_{2peak}$ with an average of 12-26%. Furthermore, 75% of the patients reached the $HR_{peak}$ >180 bpm criterion and only 25% of the patients reached the $HR_{peak}$ > 95% of age predicted criterion. It seems that the presence of ventilatory constraints in individual patients with CF limits the heart rate to increase to maximal levels during CPET. In addition, a clinically important (>9%) rise in $VO2_{peak}$ with the supramaximal verification was found in 3 out of 14 patients.[1] Analysis of our verification data on individual level revealed a potentially clinically important increase (>9% rise in $VO_{2peak}$) with the verification procedure in a comparable 4 out of 13 patients. On the other hand, in both studies, 6-7 individuals also presented with a decrease in $VO_{2peak}$ with the supramaximal verification. So, the validity of current used supramaximal verification procedures remains questionable. This result highlights the importance of using other criteria to define maximal effort in patients with CF, and the development of other strategies to increase the probability of a maximal effort and/or to verify a maximal effort in individual patients with CF. This is clinical relevant when the outcomes on the maximal exercise test are used for personalized exercise prescription or prognostics.

In addition to the previous paragraph, we developed and validated an individualized protocol for workload increments during bicycle exercise testing. The individualized exercise testing approach will provide more optimal CF-specific exercise testing of comparable test duration across a wide variety of ages and disease states. The use of individualized exercise test procedures increases the probability of achieving a maximal effort during exercise testing which was reported by Karila et al.[2]

However, in the absence of possibilities to directly measure $VO_{2peak}$ with gas analysis, a pallet of reliable and valid alternative strategies to measure or estimate $VO_{peak}$ should be available for the clinician. Regrettably, a commonly used and easy to perform field-test (6-minute walk test) is not very well associated with $VO_{2peak}$ in children and adolescents with CF.[3] Therefore, we developed a model to predict $VO_{peak}$. Using this model, it is possible, even without gas analysis, to estimate peak oxygen uptake adequately in adolescents with CF using only a cycle ergometer. Besides, the $VO_{2peak}$ estimated with this model can discriminate patients with CF in different prognosis clusters. Furthermore, responses to exercise training interventions depend on initial fitness level.[4] Therefore, using the estimated $VO_{2peak}$, patients who will benefit most from exercise training interventions (patients with low and middle fitness levels, independent of lung function) can be identified in advance.

Nevertheless, direct measurement of the $VO_{2peak}$ with gas analysis still provides additional valuable information. Breath by breath gas analysis during exercise enables assessment

of mechanical ventilatory constraints, breathing strategies and ventilatory responses during exercise in children with CF.[5] Moreover, besides $VO_{2peak}$, other prognostic markers of mortality as peak minute ventilation (VE) and the ventilatory equivalent for oxygen ($VE/VO_2$) can be determined.[6] Furthermore, the rate of lung function decline over time is significantly predicted by the presence of $CO_2$ retention during exercise (end-tidal $PCO_2$ rise > 5mmHg during exercise and failure to reduce end-tidal $PCO_2$ >3mmHg after termination).[7] This clinically valuable information lacks in the absence of gas analysis.

## Exercise limiting mechanisms

It is of clinical importance to expand current knowledge on physiologic mechanisms limiting exercise capacity in patients with CF. Ventilatory limitation (defined as: $FEV_{1\%pred}$ < 80% and Breathing Reserve index at peak exercise > 0.7 and $VO_{2peak}$ < $85\%_{pred}$) can be an important exercise limiting factor in patients with CF. Nevertheless, both $FEV_{1\%pred}$ and RV/TLC (%) were demonstrated to be significantly but only weakly associated with the presence of ventilatory limitation or exercise capacity ($VO_{2peak}$ and $W_{peak}$). This means that, besides $FEV_1$ and hyperinflation, other factors such as skeletal muscle function are also important parameters of exercise capacity in patients with CF.

The association between RV/TLC (%) and $FEV_{1\%pred}$ with exercise capacity in children with CF is confirmed by Sovtic et al.[8] They also found a significant association between RV/TLC and oxygen saturation at $W_{peak}$. Moreover, exercise induced hypoxemia and clinically important desaturation ($SpO_2$ < 90%) at peak exercise were only found in patients with static hyperinflation and ventilatory limitation. Clinically, this would implicate that all patients who present with static hyperinflation and ventilatory limitation may require oxygen saturation monitoring during exercise testing and training.[8]

A point to address is that care should be taken for the assessment and definition of a ventilatory limitation. Strategies currently used to define ventilatory limitation do not seem to be valid in patients with CF.[9] In line with these studies, we observe peak minute ventilation values exceeding calculated maximal voluntary ventilation during maximal exercise testing, even when no ventilatory limitation is expected. Besides, the prediction equation to calculate the maximal voluntary ventilation (35-40 x $FEV_1$ (l)) may not be fully appropriate in the presence of respiratory muscle weakness.[10] Furthermore, mechanical properties of the lungs do not seem to limit exercise capacity in patients with CF with normal spirometry. Expiratory flow limitation during exercise just becomes manifest in patients with more severe lung disease.[11] Measuring tidal breathing flow-volume loops during exercise and plotting them into maximal pre-exercise flow-volume loops provides extra information about the sources and degree of the ventilatory constraints.[12]

Work of breathing and, subsequently, oxygen consumption by the respiratory muscles can be increased with hyperinflation.[13] Oxygen cost of breathing during exercise is reflected

by the $VO_2$ per unit work rate ($\Delta O_2/\Delta W$), and has been shown to be higher in patients with CF.[14] The calculation of the $\Delta O_2/\Delta W$ requires the $VO_2$ during unloaded/reference cycling. Unfortunately, this parameter was lacking in the database, so this parameter could not be calculated and no insight could be given in oxygen cost of breathing during exercise in static hyperinflated versus non-static hyperinflated patients.

In contrast to previous studies,[15-17] no evidence was found that exercise capacity in clinically stable patients with CF is related to skeletal muscle mitochondrial function and/or oxygenation. This finding shows that patients with CF in a stable clinical status can be trained normally and there seems to be no skeletal muscle metabolic constraint to benefit from exercise training in clinically stable patients with CF with a good lung function. However, oxygenation can still be impeded by reduced bloodflow to the peripheral skeletal muscles during exercise with excessive ventilatory demands.[18] As described in this thesis, this could have clinical consequences for patients with CF with an increased work of breathing. In the study presented in Chapter 6 only patients with preserved lung function ($FEV_{1\%pred}$ 92.8±14.6%) were included. $FEV_{1\%pred}$ and exercise induced dynamic hyperinflation (an increase in inspiratory capacity > 100 mL) are significantly related.[19] Lower $FEV_{1\%pred}$ values were found in patients with dynamic hyperinflation compared to patients without dynamic hyperinflation ($FEV_{1\%pred}$ 66±19 versus 79±18 respectively).[19] As dynamic hyperinflation increases work of breathing,[13] it is important for future research to study oxygenation kinetics during exercise in patients with CF with higher ventilatory demands, i.e. in patients with $FEV_{1\%pred}$ < 60%pred.

## Exercise training

There is strong evidence that regular exercise has the potential to attenuate the annual decrease in lung function ($FEV_1$) in the short and long term in patients with CF.[20] Therefore, optimizing the effects of general exercise training in patients with CF is clinically important.

As $VO_{2peak}$ is decreased in the presence of static hyperinflation, an elevated work of breathing could still hamper exercise capacity and trainability in patients with CF. This was illustrated by the effect of positive-pressure proportional assist ventilation (PAV) during heavy exercise, which reduced work of breathing (-40-50%) and oxygen cost of breathing (-10-15%) in healthy males.[21] In patients with CF, continuous positive airway pressure (CPAP) decreased work of breathing and improved exercise tolerance, which was more pronounced with increasing disease severity.[22] So there seems to be an interrelationship between the work of breathing, exercise-induced diaphragmatic fatigue and exercise tolerance.[21] This highlights the clinical relevance to aim for a reduction in work of breathing.

Regrettably, a six-weeks, home-based, non-supervised IMT program did not significantly decrease work of breathing in patients with moderate CF compared to control patients. Also, a six weeks, non-supervised, 5BX training program was insufficiently effective to increase exercise capacity significantly in mild-moderate patients with CF. However, a post-hoc subgroup analysis showed a significant increase in $VO_{2peak}$/kg after general exercise training in more deconditioned patients ($VO_{2peak}$kg < 32 ml/min/kg). Indeed, the increase in $VO_{2peak}$/kg is dependent on the initial fitness level with a more pronounced effect in patients with moderate (81-58%pred) and low (<58%pred) $VO_{2peak}$.[4]

The absence of significant changes over time between the training group and controls after IMT and/or peripheral muscle exercise training could be explained by the short-term, home-based and non-supervised regime. Quality (intensity) and quantity of the unsupervised training sessions could not sufficiently be controlled. A randomized controlled trial of in-hospital, supervised training found a significant effect for combined IMT and general exercise training on inspiratory muscle strength and exercise capacity. [23] An observational study of a supervised but outpatient exercise program in children with CF also increased exercise tolerance, reduced intravenous antibiotic days and tended to reduce lung function decline within a one year follow-up.[24] These results could implicate that supervision, more than location, is a key factor in the success of an exercise intervention in patients with CF. Also, the cost of IV antibiotics was reduced with 25% compared to the preceding year with less exercise (-130 minutes exercise/week). This evident cost reduction would suggest that supervised exercise sessions and counseling by a physiotherapist (~every two weeks) are likely to be cost beneficial.[24]

Importantly, although rates of annual decline in $VO_{2peak}$ [25, 26] are available for patients with CF, caution must be taken to extrapolate these rates to shorter follow-up periods. Seasonal influences are associated with Pseudomonas aeruginosa acquisition in young children with CF [27] and Pseudomonas aeruginosa colonization might affect both the course of exercise capacity [26] and trainability [28] within the follow-up period. We suggest to use longer (>one year) follow-up periods in future exercise studies to be able to detect long-term course changes in $VO_{2peak}$ and $FEV_1$.

Additionally, the use of a home-based, unsupervised exercise regime might have decreased the overall adherence in the exercise study described in Chapter 7. Adequate adherence is, besides adequate training parameters such as frequency, intensity, time and type (FITT),[29] important to maximize training benefits in patients with CF.[30] During the intervention study described in this thesis we had several dropouts due to low adherence or underachievement. Low adherence numbers are also reported in other studies of exercise interventions in patients with CF (~40-55% of exercise sessions completed).[31, 32] Adherence is negatively associated with perceived disease severity, but seems not to be related to treatment burden of the exercise interventions separately.[31] However, the

exact theorem to understand adherence in patients with CF remains unknown.[32]
In conclusion, based on our results we recommend that future exercise studies use long-term (>12 months), supervised and more intensive training programs.

## GENERALIZATION OF THE FINDINGS

Both the model to estimate $VO_{2peak}$ and the model to predict $W_{peak}$ were developed and validated in clinically stable ($FEV_1$%pred ~87±18% [37-147%] adolescents with CF (age ~14±2 years [12-18 years]) with a broad range in $VO_{2peak}$/kg [~23 ml/min/kg - ~50 ml/min/kg], making both models clinically applicable in adolescents with mild to moderate CF, independent of measured $VO_{2peak}$. Furthermore, the measured $VO_{peak}$ on group level used to develop these models can be reliably set as the true $VO_{2peak}$ as the verification study was performed in a sample of comparable patients with CF (Age 14±2 years [12-17]; $FEV_1$%pred 81±22 [45-117]).
The intervention study was performed in a more divergent group of patients with CF, varying from 12 to 37 years of age (median 15 years) and with a mean $FEV_1\%_{pred}$ of 77% ranging from 37 to 117%$_{pred}$ (median 81%$_{pred}$). This range makes the clinical implications of this study quite generalizable on clinical stable patients with mild-moderate CF.

## CLINICAL IMPLICATIONS

Exercise capacity ($VO_{2peak}$) in adolescents with CF starts to decrease from the age of twelve.[26] $VO_{2peak}$ is a significant and strong predictor of eight year survival in patients with CF.[25, 33] Survival rates are higher in patients with the highest $VO_{2peak}$ (≥82%pred), as compared with $VO_{2peak}$ 59-81%pred and $VO_{2peak}$≤58%pred, respectively.[33] Ventilatory constraints and skeletal muscle function do not seem to play a dominant role in clinical stable, mild-moderate adolescent patients with CF with a preserved lung function ($FEV_1$ ~ 83±21%pred and RV/TLC ~32.5±10.9) (this thesis). As previously addressed, maximal exercise capacity is generally not ventilatory limited until the $FEV_1$ is less than 60%pred. [34]
We recommend performing one CPET with gas analysis before the age of ten with an annual follow-up from the age of twelve. This enables caregivers to identify those patients who are at risk for poorer prognosis, which gives the opportunity to aim and institute interventions as exercise training and/or medication.
In adolescents with a relative good lung function ($FEV_1$>60%pred) and in the absence of possibilities to formally asses $VO_{2peak}$, the annual exercise test can be performed without

gas analysis using the equation presented in Chapter 4. To increase the probability of a maximal effort, we suggest using the individualized protocol for workload increments presented in Chapter 3. As several exercise limiting mechanisms, such as ventilatory constraints, become manifest when $FEV_1$ falls below 60%pred, we recommend performing a CPET with gas analysis in patients with an $FEV_1 \leq 60\%$pred. Furthermore, when $VO_{2peak}$ decreases below 82%pred,[33] we also recommend using gas analysis to provide a more detailed analysis of the possible exercise limiting mechanisms.
When properly screened for possible contra-indications, exercise should be encouraged in every patient with CF. However, when $VO_{2peak}$ decreases below 82%pred, we recommend to start long-term (>one year), (partially) supervised exercise programs which can be performed outside the hospital. The frequency of supervision can be started weekly, decreasing to longer time-interval. Training progression and intensity can be monitored during the supervised sessions without gas analysis using the individualized CF protocol (this thesis). Proper measurements of oxygen saturation and heart rate are strongly recommended.

## FUTURE RESEARCH DIRECTIONS

The results and discussions of the studies presented in this thesis give rise to a number of debates that should be addressed in future research. First, as previously addressed, measuring flow-volume curves during exercise [35] could provide extra information about the breathing pattern and ventilatory constraints during exercise in patients with CF. Flow-volume curves during exercise are recommended for future studies to assess (the degree of) ventilatory limitation. Interestingly, performing flow-volume curves during exercise does not modify the main cardioventilatory parameters during CPET.[36]
Second, regrettably, to our knowledge, no longitudinal studies are available on these natural courses and short-term changes due to pulmonary exacerbations. This makes it difficult to compare the observed trends in Chapter 7 with normal courses. Therefore, it would be interesting to investigate the short and long-term course of the work of breathing in patients with CF. Future studies should focus on the longitudinal short and long-term course of the work of breathing in patients with CF in a wide range of disease status.
Third, in order to optimize the effects of exercise interventions, improving adherence is a valuable target to aim for. Future research should focus on the development of strategies to improve adherence to exercise interventions and exercise intervention studies in patients with CF. Qualitative studies in this area might be helpful to understand exercise adherence in patients with CF.[37]

Finally, another way to optimize training benefits in patients with CF might be the decrease of the ventilatory load and demand during exercise training. Unloading the respiratory muscles during general exercise training might be beneficial in patients with CF with increased ventilatory loads. Decreasing the sensation of dyspnea and improving oxygenation might thereby increase the sustainable and tolerated peripheral training load.[38, 39] As there is no clinical study in ventilatory limited patients with CF providing evidence to support this exercise strategy, research in this area is needed.

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## SAMENVATTING

Sinds de jaren 70 wordt er veel onderzoek gedaan naar de effecten van fysieke training bij patiënten met Cystic Fibrosis (CF). Daarnaast wordt er de laatste tien jaar veel aandacht besteed aan de rol van het meten en monitoren van het inspanningsvermogen van patiënten met CF. In de literatuur bestaat nog geen consensus over de beste methodiek om het inspanningsvermogen te meten. Verder verschillen de inzichten over de meest dominante inspanningslimiterende mechanismen bij CF en lopen de ideeën uiteen hoe trainingsinterventies op individueel niveau in te stellen.

Het doel van dit proefschift is daarom om op het domein van de CF zorg meer inzicht te geven in: [1] alternatieve inspanningstestprocedures; [2] inspanningslimiterende mechanismen; en [3] training van de inspiratiemusculatuur en perifere skeletspiermusculatuur.

Hoofdstuk 2 beschrijft de verificatie van het maximaal zuurstofopnamevermogen ($VO_{2piek}$) gemeten met een standaard maximale inspanningstest (MIT) met gasanalyse, ECG registratie en onder monitoring van zuurstofsaturatie (SpO2). De MIT werd na een herstelfase gevolgd door een supramaximaal testprotocol. Het bleek dat er op groepsniveau geen significant verschil bestond in de $VO_{2piek}$ tussen de MIT en het supramaximale protocol. Dit impliceert dat de $VO_{2piek}$ gemeten met de standaard MIT de echte $VO_{2piek}$ weergeeft.

In hoofdstuk 3 wordt beschreven hoe een individueel inspanningsprotocol is ontwikkeld. Met behulp van lineaire regressie analyse werd een model verkregen waarmee met leeftijd, geslacht, longfunctie ($FEV_1$ (L.min)) en lengte het maximaal gefietst vermogen ($W_{piek}$) bij de MIT kon worden voorspeld. De voorspelde $W_{piek}$ werd vervolgens gebruikt om de stapgrootte in oploop van weerstand tijdens de fietstest te bepalen met als streven de testpersoon in ~10 minuten het maximale inspanningsvermogen te doen bereiken. Dit individuele protocol bleek meer doelmatig ten aanzien van het bereiken van een maximale inspanning binnen ~10 minuten dan het standaard gebruikte Godfrey protocol.

Vervolgens word in hoofdstuk 4 de ontwikkeling en vervolgens de validatie van een model gepresenteerd waarmee met de $W_{piek}$, verkregen met een MIT volgens het Godfrey protocol, en geslacht de $VO_{2piek}$ betrouwbaar geschat kon worden. Met behulp van de geschatte $VO_{2piek}$ konden patiënten tevens betrouwbaard worden onderverdeeld in verschillende prognostische clusters. Dit model is klinisch waardevol, wanneer er geen mogelijkheid is om de $VO_{2piek}$ te bepalen met gasanalyse.

In hoofdstuk 5 wordt gekeken naar de voorspellende waarde van de mate van statische hyperinflatie ten aanzien van een ventilatoir beperkt inspanningsvermogen. De voorspelende waarde bleek, ofschoon statistisch significant, zwak. Verder bleek de associatie tussen de mate van statische hyperinflatie en inspanningsvariabelen licht sterker dan die van de $FEV_1$ met inspanningsvariabelen.

Patiënten met CF hebben een beperkt inspanningsvermogen. Verschillende studies laten zien dat er een samenhang is tussen longfunctie, spiermassa, energieverbruik, (adem)spierfunctie en inspanningsvermogen bij patiënten met CF. Tot op heden is er geen wetenschappelijk bewijs of overeenstemming m.b.t. het feit of de genoemde spierdysfunctie veroorzaakt wordt door een afwijking in het energiemetabolisme van de spier zelf en/of dat de spierdysfunctie wordt veroorzaakt door een verminderde zuurstoftoevoer naar de spieren. De verminderde zuurstoftoevoer naar de spieren zou veroorzaakt kunnen worden door een verhoogde zuurstofconsumptie van de ademspieren tijdens inspanning. De vraag blijft dus of er sprake is van een zuurstofaanbod probleem of een zuurstofconsumptie probleem. Hoofdstuk 6 beschrijft een studie waarin bij klinisch stabiele, milde, patiënten met CF en gezonde, gematchte controlepersonen in rust en tijdens (maximale) inspanning werd gekeken naar het oxidatief vermogen van de skeletspier enerzijds en de oxygenatie van de skeletspier anderzijds. Er bleek geen verschil in oxidatief vermogen en oxygenatie tijdens maximale inspanning en tijdens herstel tussen patiënten met CF en gezonde controlepersonen. Dit impliceert dat klinisch stabiele milde patiënten met CF normaal trainbaar zijn en dat er geen oxidatieve restrictie lijkt te zijn om positief te reageren op een trainingsinterventie.
Vervolgens wordt in hoofdstuk 7 gekeken naar twee trainingsinterventies. Ten gevolge van de continue luchtwegobstructie bij cystic fibrosis (CF) ontstaat een chronische hyperinflatiestand van de thorax, waardoor het diafragma minder efficiënt gaat werken en waardoor de ademarbeid verhoogd wordt. De verhoogde ademarbeid veroorzaakt een zogenaamde metaboreflex met als gevolg een vasoconstrictie van de bloedvaten in de (inspannende) skeletspieren. Het is aannemelijk dat deze verminderde bloedtoevoer naar de skeletspieren het inspanningsvermogen negatief beïnvloed waardoor patiënten in het dagelijks leven minder kunnen doen dan gewenst. De ademarbeid beïnvloeden door training van de inademmusculatuur (IMT) zou het inspanningsvermogen kunnen verbeteren. Daarmee zou een ongesuperviseerd perifeer spiertrainingsprogramma (5BX) meer effect hebben, wanneer er gepreconditioneerd is met IMT. Uit de studie bleek dat het effect van IMT op de ademarbeid niet verschilde tussen de placebogroep en de trainingsgroep. Binnen de trainingsgroep bleek de ademspierkracht echter significant toe te nemen en nam daarmee nam de ademarbeid af. Verder bleek dat het effect op het inspanningsvermogen van het ongesuperviseerde 5BX programma niet verschilde tussen de groepen. Het is echter klinisch zeer interessant dat het inspanningsvermogen wel statistisch significant verbeterde in een subgroep van zwaar gedeconditioneerde patiënten. De vraagstelling m.b.t. een mogelijk preconditionerend effect van IMT kon met deze resultaten echter niet worden beantwoord.

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# CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 11 februari 1981 te Ede (GLD). Hij volgende het middelbaar onderwijs (VWO) aan het Christelijk Lyceum Veenendaal en behaalde het eindexamen in 1999. Aansluitend ronde hij de studie Fysiotherapie aan de Hogeschool voor Fysiotherapie Thim van der Laan te Utrecht af in 2001. Vervolgens ronde hij de studie Gezondheidswetenschappen, afstudeerrichting Bewegingswetenschappen af aan de Universiteit van Maastricht in 2005.
Na diverse banen als fysiotherapeut in de 1e lijn startte hij in oktober 2008 met zijn promotieonderzoek onder leiding van Prof. Dr. P.J.M. Helders van het Kinder-bewegingscentrum van het Wilhelmina Kinderziekenhuis, Universitair Medisch Centrum Utrecht. De resultaten van dit promotieonderzoek zijn beschreven in dit proefschrift. Daarnaast is hij sinds december 2007 werkzaam als fysiotherapeut bij Fysio Sport & Training 'De Isselt' te Amersfoort.

# LIST OF PUBLICATIONS

## Publications

- Hulzebos HJ, **Werkman MS**, Brussel van M, Takken T. T. Towards an individualized protocol for workload increments in cardiopulmonary exercise testing in children and adolescents with Cystic Fibrosis. J Cyst Fibros 2012; 11(6):550-554.
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## Abstracts

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Oral presentation North American CF Conference Salt Lake City, october 2013; Oxidative skeletal muscle metabolism during exercise in adolescents with CF.

Presentatie KNGF Congres 2013; Ademspiertraining voorafgaand aan algemene training bij patiënten met Cystic Fibrosis.